Botanical

Volume LXXXIII Number 4

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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN-KABUSHIKI-KAISHA, Tokyo, Osaka, Kyoto, Fukuoka, Sendai
THE COMMERCIAL PRESS, LIMITED, Shanghai

Volume LXXXIII Number 4

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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Issued June 22, 1927

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, Canary Islands, El Salvador, Argentina, Bolivia, Brazil, Colombia, Chile, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Hayti, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Balearic Islands, and Spain. ¶Postage is charged extra as follows: For Canada and Newfoundland, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, £2 8*s*. each; single copies, including postage, 6*s*. 8*d*. each.

For China: The Commercial Press, Limited, Paoshon Road, Shanghai. Yearly subscriptions, including postage, $9.60; single copies, including postage, $1.33, or their equivalents in Chinese money.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

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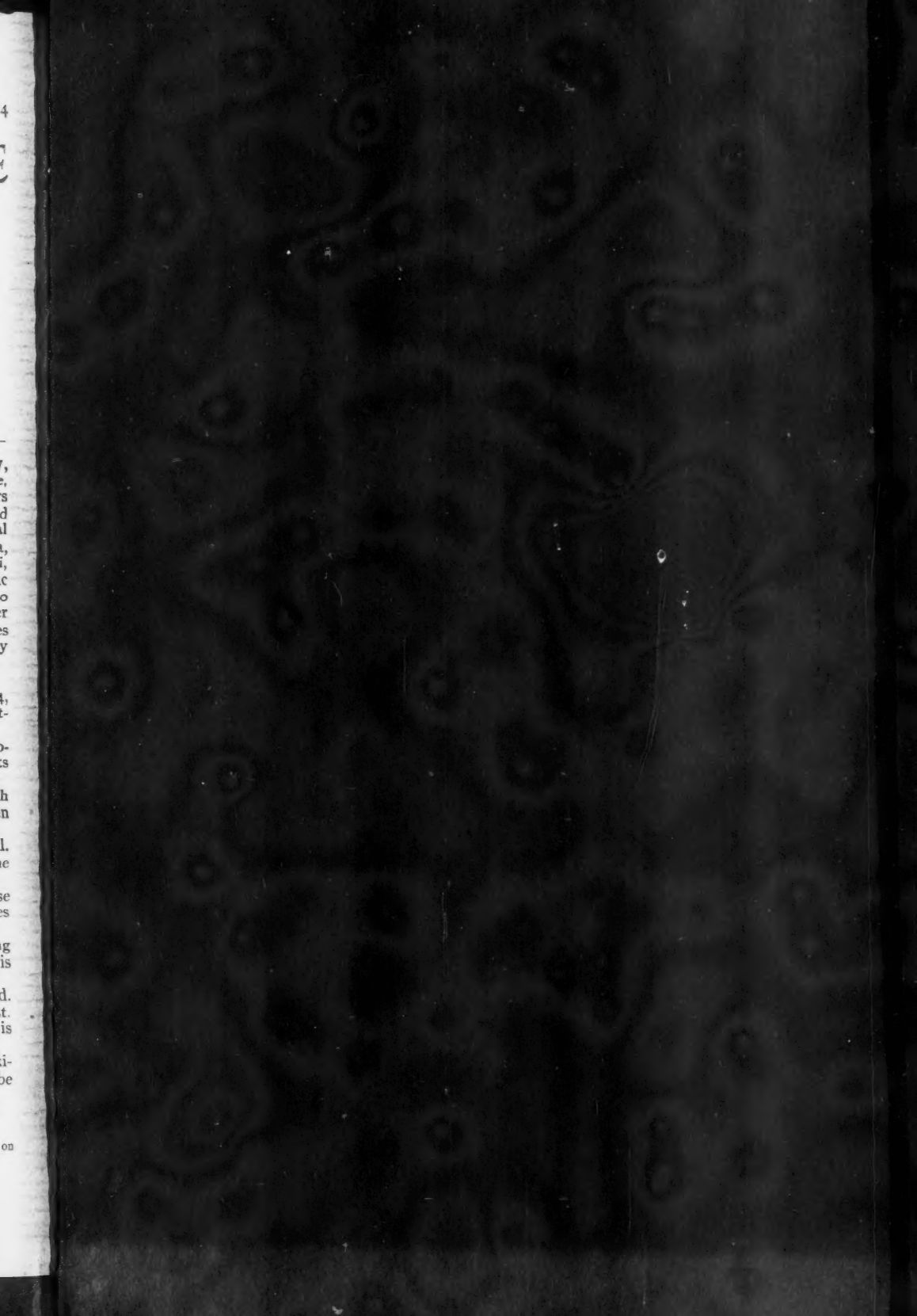
Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15 1918.

PRINTED IN THE U.S.A.



The PRESS IMPRINT

June and July 1927

Humanizing the Sciences

Comment on *The Nature of the World and of Man* by sixteen members of the Faculty of the University of Chicago is running high.

Whatever their intentions toward college Freshmen, which are of no very great interest to the world outside the universities, the sixteen scientists have written sixteen fireside lectures ranging from astronomy to psychology, which the critics rank as the best of recent attempts to humanize the sciences. Opinion runs as follows:

"One of the finest and most engrossing popularizations of current science in existence. . . . . It is simple, clear, concrete, reliable. One emerges from it the richer by solid information and definite concepts."—HENRY HAZLITT, *New York Sun*.

"A manual of the physical and biological sciences of which it would be hard to speak too highly. The volume is so many things that an outline ought to be but frequently is not. It is selective in the sense of plucking the real heart out of a body of data."—*New York Times Book Review*.

"It is unexcelled for the general reader and many of those who have had scientific training will here learn for the first time what it is all about. Above all, it is vigorously inspiring."—*New York Herald-Tribune*.

"For the story is well told, well illustrated, and well colored with human significance. Certainly the volume is accurate; the names of the various contributors guarantee that. Certainly it is interesting and readable, and popular without being diluted."—*The Nation*.

"It sweeps on from one great truth to another in a way that gives one a profound respect for the recent progress of science."—*Saturday Review of Literature*.

"It is fascinating reading for anyone who has a spark of that divine curiosity about the ways of life which causes man to seek, and seeking, to progress."—*Chicago Tribune*.

That the orientation course upon which *The Nature of the World and of Man* is based is helping the students build a workable philosophy of life is indicated in a large body of comment from these students themselves. The following statements are representative of the student attitude toward the course:

"To me this course has been most stimulating. It has given a new and broader conception of the universe. It has aroused in me a new interest and spirit of curiosity. I feel that to some extent, I have learned to value the scientific spirit. The influence that science has upon our lives is better appreciated. I believe that the course was intended to present a brief but comprehensive picture of everything that concerns us, to shake up our minds, and to stimulate us to a little thought. It has done those things for me. I am eager to take more science courses and find out how and why the universe is as it is."

"I find the spirit of science to be open-minded, strong, fearless, and wholesome. It is strange to consider one's self related to the great universe of things. I am very enthusiastic about the course. The realization or the apprehension of the infinitude of the heavens, the universe of the atom has put me in my proper place in the cosmos."

"This course is called an orientation course. This expresses well its aim and what it has

done for me. It has taken me out of the universe and has set me at a point outside where I could see everything that was going on and look at the ways of the universe from an impartial perspective. It has given an introduction to the natural sciences and their interrelationship. The greatest benefit I have received, a benefit which sinks all other results into insignificance, is that it has caused me to ask myself such questions as 'Who am I?' 'What am I?' and 'Where am I?' It has changed my outlook on the world."

Two printings of *The Nature of the World and of Man* have already been exhausted and a new printing of 15,000 has been ordered. This will include a revision of the chapter on "The Nature of Chemical Processes" by Julius Stieglitz. As re-written it will simplify the subject of chemistry for the layman. *THE NATURE OF THE WORLD AND OF MAN.* By Sixteen Members of the Faculty of the University of Chicago. *$4.00, postpaid $4.15.*

* * *

Gangs

Gang activities are familiar enough in cosmopolitan cities, but strangely, there has been no adequate sociological study of them. The forces that explain gangdom, adult and juvenile, have been brought to light by Professor Frederic M. Thrasher in *The Gang,* a book which critics believe destined to become the standard source book on the subject.

Professor Thrasher spent six years in his study of gangland—the poverty belt which surrounds Chicago's loop district. He lived with the gangsters, interviewed their leaders, and became intimately acquainted with gang life from the inside. Mingling with the "Murderers," the "Deadshots," and members of hundreds of other gangs, he learned their mysteries and secret signs, their attitudes toward society, and toward each other. And he found in investigating 1,313 gangs of all ages and sizes, the facts which explain the sensational features of gang life which are flaunted in the headlines.

He looks upon the gang as a social makeshift, which naturally appears in the cracks of the community and inevitably fosters the spread of crime. Abandoning the idea of a gang instinct, he attempts through a careful study of the natural history, structure, and functioning of the gang, to arrive at the true causes for its existence.

Mr. Thrasher has collected a vast amount of intensely interesting material which he discusses with a minimum of seminar jargon. A large part of the case material was secured from the gang boys themselves. A typical case is the following, obtained in an interview with a gang boy:

Billy, the Brains

Billy was the brains of the gang. He was "educated," a high-school boy too. He would work sometimes, but not often. The kids would bring their "stuff" to him. One day we had a big fight over it when we were robbing a merchandise car; we had cigarettes, pop, and a lot of other stuff. Billy had his stuff put away in a box with straw on top of it. The watchman looked at the straw, but Billy told him it was for a rabbit. Billy would sell "cartoons" (of cigarettes) for a half a dollar apiece.

Billy would plan things for our gang. He would get us a place to sleep when we were bumming away from home. He would get us keys to the bread boxes, so that we could get food when we were hungry. We would get the bread after the bakers left it early in the morning before the stores opened. . . . . Billy would find us a place to sleep in some house or basement. He would go around everywhere to see if there was a place to sleep or rob; he was a regular investigator.

Henry Zorbaugh of New York University comments: "Dr. Thrasher's monograph should be of interest to the sociologist and psychologist as a model of social research and a contribution to our knowledge of human behavior; to educator and social worker as an illuminating background against which to consider the practical problems of boy adjustment; and to the romantically minded as a fascinating story of 'the web of life' as it is spun in a great city."

Harry Hansen says in the *New York World:* "Here is a remarkable document and one that ought to be a source book for years to

come. . . . . He has dug right down into boyland, gone to the root of the problem, and produced a book that will become an authority." *THE GANG*. By FREDERIC M. THRASHER. *$3.00, postpaid $3.15.*

Ernest DeWitt Burton

During a half-century of research Ernest DeWitt Burton achieved an international reputation as a New Testament scholar. In two years of extraordinary activity as President of the University of Chicago, he became equally well known as an educational executive. In addition, a leader in missionary and denominational enterprises, he offers an inspiring example of life lived to the full.

Dr. Burton's personality made a deep and lasting impression on those who knew him. "In him," says Henry Justin Smith, "were concentrated the mental vigor of youth and the benignity of age; the devotion of a missionary and the acuteness of a worldling; the simplicity of a plain man and the delicacy of a true aristocrat."

Of his associates, no one is better qualified to portray this great and enormously attractive individual than Thomas W. Goodspeed, who has been an intimate witness of his life-activity. In *Ernest DeWitt Burton: A Biography*, Mr. Goodspeed has followed his career from his student days at Denison, through his work as head of the Oriental Educational Commission, chairman of the China Educational Commission, and President of the University of Chicago, to his last days.

Mr. Goodspeed has not attempted to idealize Ernest DeWitt Burton, but to present him as he was in every period of his life. *ERNEST DeWITT BURTON*. By THOMAS W. GOODSPEED. *$3.00, postpaid $3.10.*

Dr. Burton left unpublished some exceedingly valuable work, and this has been preserved in two posthumous volumes, *Christianity in the Modern World*, and *A Short Introduction to the Gospels*.

The first of these volumes brings out one of the most attractive phases of Dr. Burton's character. Internationally known as a New Testament scholar, and as President of the University of Chicago, Ernest DeWitt Burton has another side no less great, but not as well known. This was Dr. Burton the Christian and exemplar of the practical Christian life, the man of deep religious experience and conviction.

This phase of his character is uppermost in this collection of his fugitive utterances on religious themes. Here are his great *apologia pro vita sua*, "Why I am content to be a Christian"; his views upon Jesus and Paul; and the essence of his thinking on Christian education and world-problems.

This book is fittingly concluded with "Christian Education in China," Dr. Burton's last public address.

The second of the two volumes incorporates his final conclusions in a work representing one of his most important projects. Dr. Burton continued during his last years the researches concerning the origins of the Four Gospels which had been the basis of his valued *Short Introductions to the Gospels*. He made further investigations regarding the dates of the Synoptic Gospels, and approached the Fourth Gospel from a new point of view, applying the method of source criticism which he had already used with conspicuous success in the case of the Synoptic Gospels.

These significant advances have been incorporated by Harold R. Willoughby in a new revision of the volume, which brings to biblical students valuable new material. Two important sections of fresh material found in Dr. Burton's files have been added. The first renders an exceptional judgment regarding the dates of the Synoptic Gospels. The second outlines a unique theory concerning the composition of the Fourth Gospel which illuminates the Johannine depiction of Jesus.

This authoritative revision places before students of the Gospels those facts concerning the purpose and point of view of each of them which are most necessary for an intelligent understanding of them. *CHRISTIANITY IN THE MODERN WORLD*. By ERNEST D. BURTON. *$2.00, postpaid $2.10. A SHORT INTRODUCTION TO THE GOSPELS*. By ERNEST D. BURTON AND HAROLD R. WILLOUGHBY. *$1.75, postpaid $1.85.*

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New Series of German Texts

The new "University of Chicago Junior College Series" of German texts embodies all the most recent progress in the methods

of teaching German. In fact, those who follow the articles on language pedagogy in the language journals will readily see that this series not only incorporates the best of what has been done but goes far beyond—making a new and distinct contribution of its own.

Two volumes are ready for immediate use. They are *A Modern German Grammar*, by Peter Hagboldt and F. W. Kaufmann; and *Inductive Readings in German*, Book I, by the same authors.

The grammar offers an absolutely new method of combining analysis and synthesis. Each lesson is scientifically constructed, with analysis of the reading text and development of grammatical principles followed by synthesis or actual use of what has been learned.

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* *

The "University of Chicago Junior College Series" of French texts, initiated not long ago with Otto F. Bond's *The Sounds of French* and *An Introduction to the Study of French*, has, because of the departure of its methods from those generally in use, become a center of controversy. Mr. Bond maintains that most of his critics have mistaken his aims. Answering one of them, Colley F. Sparkman, in the *Modern Language Journal*, he says: "I am afraid that Mr. Sparkman suspects me of having wished upon the public a substitute for a composition grammar or some *bête difforme* lacking synthetic legs and a *raison d'être*, since he would have me add various types of exercises involving composition. Let me point out that the Preface (page viii) states the purpose of the book as being to enable the student to read (not write or converse!) in a short time and to prepare him for better and more rapid progress in acquiring expression power.

"Incidentally, why do language teachers struggle manfully to uphold the conversation and composition banner in beginning courses, since better than 50 per cent of their beginners leave them when the requirements have been satisfied?"

* * *

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Scientific study of science teaching has had some valuable results, and these Mr. Downing summarizes in this volume. Here is shown how the growing body of facts about the pedagogy of science, which have been ascertained by the experimental method, may be applied practically to the problems of the science teacher.

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From this point Mr. Downing takes up the principles of selection and organization of science materials, and actual methods of instruction—by book, lecture-demonstration, and laboratory. There is specific information on notebooks and drawings, field trips, testing results; and suggestions are made for a complete science library in the school.

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Brains of Rats and Men

By C. JUDSON HERRICK

That man's brain has been slowly developed out of the brain structures of the lower animals; that human behavior patterns and the noblest human faculties are functions of man's peculiar protoplasmic organization—to many these still seem difficult statements to accept. Their truth, however, is further demonstrated by C. Judson Herrick in this book on the origin and biological significance of the cerebral cortex.

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lion poorly nourished children from all classes in New York City alone.

There are many reasons why not only the nutrition expert but the parent needs the best available scientific knowledge of the nutrition problem. Malnutrition is an insidious enemy of childhood—it stunts growth, shortens life, increases susceptibility to disease, retards mental development. Its effect upon the nervous system is especially marked. The rapidly developing brain and nervous system of a child require certain food materials, and a moderate covering of fat is needed to protect the nerves from external stimulation. When this is lacking, headaches, insomnia, irritability, over-activity, and even hysteria develop. If nothing is done to improve his condition, the undernourished child becomes thin, anemic, wing-shouldered, dull, listless, and backward.

In combating malnutrition, teachers, parents, students, and others concerned with nutrition problems have been handicapped by the lack of a comprehensive survey of the entire subject. The needed information has been scattered through hundreds of magazines, journals, and bulletins, many of them written in technical language. In *Nutrition Work with Children*, Lydia J. Roberts has supplied the needed survey. Here in a single volume are materials for which it was previously necessary to search through many. *NUTRITION WORK WITH CHILDREN*. By Lydia J. Roberts. *$3.50, postpaid $3.65.*

* * *

The Voice of a University

We have recently printed, and should be glad to send those who want it, a booklet about the University of Chicago Press and how it functions as "The Voice of a University." To quote from it: ". . . . there are organizations which see in books not only the beauty of form, but which see through the book to its contents, and which insist that in content the quality of the printed page shall be preserved. This is the special opportunity of the great university presses. The university presses must restore and deserve the medieval belief in the sacredness of books. If men can find in books ideas which liberate and enlighten them, their reverence for books will live. . . . . This is the special function of the university press."

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The Macmillan Company, Agents in the United States
60 Fifth Avenue, New York City

VOLUME LXXXIII NUMBER 4

THE
BOTANICAL GAZETTE

June 1927

BIOCHEMISTRY OF PLANT DISEASES

IX. PECTIC ENZYMES[1]

F. R. DAVISON[2] AND J. J. WILLAMAN

(WITH FIVE FIGURES)

I. Introduction

Pectin plays an important rôle in the life processes of plants, and for that reason the solution of some of the problems related to the pectic enzymes should throw some light on the physiological significance of pectin in plant life. The importance of these substances is shown by the fact that they are found almost as widely distributed as cellulose. Pectic compounds are essential constituents of cell walls of all the higher plants, being the main constituent of the middle lamella, and as such a cementing material between cells. It is here that the pectic substances have aroused the interest of the plant pathologist, because of the ability of certain parasites to penetrate tissues by virtue of their pectic enzymes.

The number and nature of pectic compounds are not known. Furthermore, the number of enzymes that act upon these pectins is disputed. Three of them have been described, but only two are admitted by some investigators. Our principal interest in them is from the standpoint of parasitism in plants, since the possession of one of these enzymes by a fungus may enable it to penetrate a host tissue,

[1] Published with the approval of the Director, as Paper no. 647, Journal Series, Minnesota Agricultural Experiment Station.

[2] The material in this paper was presented to the graduate school of the University of Minnesota by F. R. DAVISON, as his thesis for the degree of Doctor of Philosophy.

and the possession of another may gain for the fungus some nutriment from the pectins.

Because of the facts that pectins are so widely distributed, that they have lately been receiving considerable attention at the hands of investigators, that a knowledge of their enzymes is in a very unsatisfactory state, and that a better knowledge of them would aid in some of the problems of parasitism, it was thought desirable to initiate a study of them. The work reported here deals largely with their identity, and with the means of measuring their activity.

II. Definitions

At the present time there are generally recognized three enzymes acting upon the pectic substances. Considerable confusion exists in the literature concerning the naming of these enzymes. The names given by their discoverers are pectosinase, pectase, and pectinase. Although in general it is advisable to use names given by discoverers, in the case of the first enzyme mentioned it is believed preferable to make the terminology conform to that recommended by the committee on pectin nomenclature of the Division of Agriculture and Food Chemistry of the American Chemical Society. This also conforms more closely to the standard etymology of enzymes of using the suffix -ase with the root form of the substrate. Since the diction of enzyme literature can be no clearer than that of the substrates of the enzymes involved, it is believed to be in the interest of clarity to present the committee's definitions of both the pectic substances and their enzymes. These definitions are as follows:[3]

PROPOSED NOMENCLATURE (REVISED MARCH 8, 1926)

PECTIC SUBSTANCES.—A group designation for those complex carbohydrate derivatives which occur in plants, or are prepared from plants and which are characterized by the presence of galacturonic acid units. In the naturally occurring pectic substances, the galacturonic acid units apparently exist in an acid reacting complex associated with arabinose and galactose units. This acid complex may

[3] As a member of the committee, the junior author had access to the definitions as submitted. Until adopted by the committee on nomenclature of the society they are to be considered as tentative.

occur as a free acid or as a metallic salt, but usually occurs in ester combination with methoxyl groups.

PROTOPECTIN.—The term applied to the water insoluble, unhydrolyzed, methoxylated parent pectic substances which occur in the cell walls of plants, possibly in combination with cellulose or other plant constituents, and which on restricted hydrolysis yield pectins.

PECTIN.—The term applied to the water soluble, methoxylated pectic substances occurring in plant tissues, or to the methoxylated pectic substances obtained by restricted hydrolysis of the protopectin, so regulated as to produce maximum solution of pectic substance with a minimum cleavage of methoxyl. The product may be a mixture of substances of varying methoxyl content; the term pectin or pectins is accordingly a group designation for all intermediate pectic substances between protopectin and pectic acid. It is proper, however, to refer to an individual of the group as a pectin.

PECTIC ACID.—The term applied to the water insoluble pectic substances obtained by the hydrolysis of pectin with the complete elimination of the methoxyl groups. Pectic acid may be variable in composition, according to the type of hydrolysis employed; accordingly there may be a number of pectic acids, all of which are methoxyl free.

PROTOPECTINASE.—The term applied to the enzyme which hydrolyzes or dissolves protopectin, with the resultant separation of the plant cells from each other, usually spoken of as maceration. Presumably the product of this hydrolysis is pectin. The term protopectinase supersedes the older term pectosinase with which it is synonymous.

PECTASE.—The term applied to the enzyme which converts pectin into pectic acid, the latter becoming a gel, especially in the presence of calcium (or barium or strontium) salts.

PECTINASE.—The term applied to the enzyme which hydrolyzes pectin and pectic acid into their simplest soluble cleavage products, which are probably arabinose, galactose, and galacturonic acid.

The writers feel that they have proved beyond reasonable doubt the existence of the three enzymes here defined; they will therefore use this terminology throughout the present paper. Since rather

complete reviews of the literature of the pectic enzymes have recently been published (1, 39), this literature will be referred to only incidentally during the presentation of the following data.

III. Protopectinase

Occurrence and preparation

A macerating action on plant tissue has been reported for many species of bacteria and fungi (2, 3, 4, 24, 26, 29, 31, 37, 42). Recently protopectinase production has been the subject of extensive researches by Harter and Weimer (16–21), although they call it pectinase. They found that this enzyme is produced by several species of *Rhizopus*, that some of it is retained in the mycelium, and that a portion is excreted into the substratum. They also found it in the spores of *R. nigricans* and *R. tritici*. Miss Paton found this enzyme (called by her pectinase) in the eighteen species of pollen that she studied, and a histological examination showed that pollen tubes make their way between the adjacent cells rather than through them. Spaulding (34) observed that in the last stages of wood decay produced by *Lenzites saepiaria* Fr. the middle lamella has disappeared. Zeller (43) found that this same fungus produces a substance dissolving the middle lamella of carrots and potato disks, coherence of the tissue being lost after 42 hours in an extract of the fungus powder.

A number of materials prepared by one or more of these methods were tested for protopectinase. The results are given in table I, using in this case a relative index of activity instead of definite time units for maceration. It will be noticed that this enzyme was found only in materials of plant origin. Its absence from the corn pollen should not be taken as conclusive, since this pollen was 7 years old at the time of testing.

This enzyme was prepared in three different forms: (1) Powdered mycelium produced by growing the organisms *Rhizopus tritici* and *Bacillus carotovorus* upon carrot decoction for 24–48 hours, then harvesting the mycelial mat, drying and grinding; this powder was extracted with water for use. (2) The liquid medium upon which the *Rhizopus* had grown. (3) The powder formed by precipitating the

enzyme from the medium with alcohol, filtering, drying, and grinding.

Large quantities of mycelial mat were prepared by inoculating sterile 250 cc. Erlenmeyer flasks containing 25 cc. of carrot decoction with spores from the young growth of the fungus to be used. At the end of the incubation period the fungus mat was harvested and washed in running tap water for 5 minutes, the water squeezed out, and then treated with acetone and ether, according to Dox's (13)

TABLE I

OCCURRENCE OF PECTIC ENZYMES IN MATERIALS TESTED

SOURCE	PROTOPECTINASE	PECTASE	PECTINASE
Rhizopus tritici	+++*	− − −	++
Aspergillus niger	− − −	− − −	+
B. carotovorus	+++	− − −	++
Sclerotinia cinerea	− − −	+++	+++
Clover leaves	− − −	+++	− − −
Pollen (corn)	− − −	+++	− − −
Barley malt	− − −	− − −	++
Barley malt (fresh)	− − −	+(?)	++
Diastase of malt	− − −	− − −	− − −
Diamalt	− − −	− − −	− − −
Diastophore	− − −	− − −	− − −
Diazyme	− − −	− − −	− − −
Takadiastase	+	− − −	+
Emulsin	+	− − −	+
Ptyalin	− − −	− − −	− − −
Amylopsin	− − −	− − −	− − −
Steapsin	− − −	− − −	− − −

* +++ Very abundant; − absent.

modification of ALBERT and BUCHNER's method. The method is as follows. The mycelia after being washed are immersed for 10 minutes in a large excess of acetone and constantly stirred, the hyphae being torn apart by forceps at the same time. The material is then squeezed dry and immersed in a fresh supply of acetone for 2 minutes with constant stirring. It is again squeezed dry and stirred for 3 minutes in ether, after which it is dried by a fan, then over sulphuric acid in a vacuum.

QUANTITATIVE DETERMINATION

The detection of this enzyme is based upon the fact that by dissolving the pectic lamella between the cells of certain plant tissues

the cells separate, and a maceration effect is produced. The methods used heretofore are qualitative rather than quantitative. BROWN (8, 9) and HARTER and WEIMER (17) used the time required for complete loss of coherence of the tissue submerged in the enzyme extract, judging the end point with their fingers. BROWN used disks of potato tubers and turnip roots 0.5 mm. thick, and HARTER and WEIMER used disks of sweet potatoes 1 mm. thick. The writers found it very difficult to get an accurate indication of the maceration by examining the disks, so it was finally decided to use the following technique for estimating maceration. A strip of potato tissue 0.5 mm. thick, 5 mm. wide, and about 20 mm. long was cut by the aid of a microtome and a knife made by clamping two safety razor blades over lead slugs the proper distance apart. By keeping the instruments very sharp, and taking slices very close together, tissue of uniform thickness and texture could readily be obtained. A strip thus cut was suspended by folding over the ends and fastening a small paper clamp on each end. Considerable care must be exercised in cutting the potato strips, and also in fastening the clamp so that there is a direct pull downward. A 10 gm. weight or its equivalent was hooked on one end, and the tissue and weight suspended in a test tube containing 10 cc. of the extract to be tested, and the whole placed in a thermostat. The time when the tissue disintegrated and the weight fell was noted. This is believed to be more accurate and to give more absolute results than the other methods mentioned.

IDENTIFICATION

In the list of materials in table I, it may be noted that *Sclerotinia cinerea*, the fungus causing the brown rot of plums, did not show any protopectinase activity. On the basis of photomicrographs of rotted tissue, VALLEAU (36) concluded that this fungus follows the line of the middle lamella in penetrating a tissue; and from this evidence WILLAMAN (40) predicted that a protopectinase would be found in the fungus, and MUHLEMAN (28) reported positive evidence of its presence. Its absence in these tests induced the writers to make a more careful search for it.

The pure culture of *Sclerotinia cinerea* was obtained from Dr. LOUISE DOSDAL of the Department of Plant Pathology of the Uni-

versity of Minnesota. Soaked dried peaches were used as a medium for the production of a supply of spores. For the culture medium an extract was prepared by stewing dried prunes and pressing out the juice and diluting to a Brix of 12°. Thirty cc. of this juice was placed in several 250 cc. Erlenmeyer flasks and autoclaved at 15 pounds for 20 minutes, cooled, inoculated with spores, and placed in an incubator at 25° C. for 2 days. The mycelia were then harvested, washed, and dried by DOX's method, and then dried in a vacuum over sulphuric acid. A 1 to 2 per cent extract was tested for its macerating activity qualitatively, by its macerating effect on potato and apple disks. No macerating activity could be detected.

CONDITIONS FOR GROWTH

The conditions for growth and maceration were then carefully studied to determine if possible the presence of a macerating enzyme, and the conditions necessary for its maximum production.

The hydrogen-ion concentration of the prune juice was adjusted according to the method employed by KARRER and WEBB (**25**). The most luxuriant growth was found at a P_H ranging between 3.5 and 6.5.

The effect of the concentration of the prune juice on mycelial growth was then studied. Several flasks in duplicate were adjusted to a Brix ranging from 1°–22°. These flasks were sterilized carefully and inoculated with *Sclerotinia cinerea*. The best growth was found between 7° and 18°, with no definite optimum.

A number of materials were tested to see which supported the most luxuriant growth of spores in the shortest time. The results are listed in table II. Peaches, pears, and apricots were soaked over night, peeled, and placed in Petri dishes and sterilized. After cooling they were then inoculated with the organism. From the results in table II it is seen that the best material for the propagation of spores is prune juice.

It was thought that lowering the osmotic pressure of the medium might improve the growth of the fungus. Peaches were therefore soaked in successive quantities of distilled water for 3 days previous to sterilizing. This had little effect on the growth; if anything it hindered it slightly.

Using prune juice as a medium and adjusting its acidity and density for maximum growth of the fungus, a great number of species of *Sclerotinia* and the closely related *Monilia* were tested for protopectinase activity. Most of these were obtained from DR. E. E. HONEY of Cornell University, who very kindly sent the cultures and a list of their origins. The list is given in table III. In addition plum mummies were obtained during the winter from University Farm and the Fruit Breeding Farm at Excelsior, Minnesota. These mummies were treated with 50 per cent alcohol to remove air bubbles, and the outside layer sterilized 2 minutes in 1:1000 mercuric chloride, then washed with sterilized water and placed in a moist chamber. A good growth of spores of *S. cinerea* was formed after several days, and a pure culture was easily procured.

TABLE II

SPORE PRODUCTION OF SCLEROTINIA CINEREA ON VARIOUS MEDIA

MEDIUM	RELATIVE SPORULATION	MEDIUM	RELATIVE SPORULATION
Potato dextrose agar	+	Potatoes (mashed)	+
Prune agar	+	Sweet potatoes (mashed)	+
Peaches (soaked) first sample	+++	Carrot (mashed)	+
Peaches (soaked) second sample	+++	Carrot extract	+
Peaches (soaked) third sample	+++	Beer wort	+
Pure agar	−	Peach extract	++
Pears (soaked)	+	Apricot extract	++
Apricots	+	Prune extract	++++

In all cases the mycelia were harvested as soon as they covered the surface of the medium, usually 2 days. Extracts were made as noted, and tested for protopectinase. In none of the fungi could any trace of the enzyme be detected.

Since mycelia 2 or 3 days old showed no protopectinase, the procedure adopted by BROWN (8) was tried. He made a very thick suspension of spores in a nutrient medium, and allowed them to germinate just 24 hours. A heavy spore suspension was obtained by inoculation of sterile soaked dried peaches. A strong suspension of these spores was made by adding sterile distilled water and rubbing the spores loose; they were then inoculated into several flasks of prune juice. Sterile conditions were adhered to as closely as possible, but little contamination could result in the time given to the growth.

After 24 hours the growths were harvested, treated by Dox's method, and then dried in a vacuum over sulphuric acid, ground, and a

TABLE III

LIST OF FUNGI SUPPLIED BY DR. E. E. HONEY AND TESTED FOR PRESENCE OF PROTOPECTINASE

CULTURE NO.	
H 193	Sclerotinia cinerea f. pruni from Dr. Wormald, East Malling, England; isolated from an ascospore; Wormald's culture no. 3995
H 194	S. cinerea f. mali from Dr. Wormald; culture no. 3989
H 201	S. cinerea from Dr. Westerdijk (Centraalbureau Schimmelkulturen), Baarn, Holland; data coming with this culture were as follows: "Sclerotinia cinerea (Bon) Schr. isolated by Miss Berkhout from Persica spec. at Baarn. This culture came in our collection in 1922"
H 202	S. cinerea from Dr. Westerdijk (Centraalbureau Schimmelkulturen), with following data: "S. cinerea (Bon) Schr. Isolated May, 1921, from Prunus cerasus at Baarn"
H 209	S. cinerea collected apothecia near Ithaca, N.Y.; made single spore isolations from the apothecia on peach mummies, April 24, 1924
H 220*c*	S. cinerea collected apothecia on plum mummies from tree of Prunus triflora (var. Abundance), University Orchard, Ithaca, N.Y., May 10, 1924; made single ascospore isolations
H 238*b*	S. cinerea (Monilia stage); single spore isolations made from conidia produced upon mummied plums, collected by Dr. E. F. Guba near Webster, N.Y., May 15, 1924; isolation by E. E. Honey
H 234	Monilia cinerea from Dr. Wormald, England, isolated from conidium
H 266	S. cinerea? causing blossom blight of apricot, collected by Mr. B. A. Rudolph, at Nicholson orchard, Aromas, Cal., and isolated by E. E. Honey from spore dilution cultures of conidia; Early Golden variety of apricot
H 205	S. laxa from Dr. Westerdijk (Centraalbureau Schimmelkulturen), with following data: "Sclerotinia laxa (Ehrenb.). We received this culture from Dr. Faes, Station federale d'essais viticoles, Lausanne, Switzerland, in March, 1924"
H 251	S. cinerea from Mr. J. C. Neill, Biological Laboratory, Wellington, New Zealand, with following data: "Cultures of Monilia stage of S. cinerea Schroet. From stock cultures originally made from diseased tissue of Prunus persica fruit. Auchland 30/1/24 collected and isolated by G. H. Cunningham"
H 295	Monilia oregonensis from Dr. H. P. Barss, Corvallis, Ore.; type called Monilia oregonensis by us; isolated from sporodochia on peach fruit, in orchard near Corvallis, January 27, 1924
H 195	S. fructigena from Dr. Wormald, England, his culture no. 3979; isolated from apple
H 323	Monilia fructigena from Dr. Wormald, isolated from plum by means of single conidium
H 233	S. fructigena from Dr. Wormald, isolated from pear by means of single conidium
H 322*e*	S. cinerea on peach from University Orchard, Ithaca, N.Y., May 3, 1925
H 234	Monilia cinerea from Dr. Wormald; cherry, April 23, 1925

strong water extract tested for protopectinase activity. Nearly half of the strains were tested in this way, but no activity could be detected.

It was thought possible that repeated growing of the various

strains of *Sclerotinia* on artificial media had caused a gradual degeneration of this enzyme; therefore an attempt was made to regenerate it. Several sound apples were cleaned and the surfaces well washed, and then placed in 1:1000 $HgCl_2$ for 2 minutes. After drying they were inoculated with spores through punctures in the skin, and placed under bell jars. Transfers were made successively through four apples. From each "generation" the fungus was isolated and tested for macerating ability. Both potato and apple tissue were used in the test, but no maceration could be detected.

BROWN (**8, 9**) found that the best reaction for maceration was the reaction of the extracts as obtained from the hyphae, and adjusting to any other P_H had no advantageous effect. He did not give the reaction that gave the maximum activity. Using extracts from *Rhizopus tritici*, the P_H was adjusted through a considerable range, as suggested by KARRER and WEBB (**25**), and checked by the potentiometric method. A blank determination was run in all cases. The data are given in fig. 1, in the top pair of curves. The greatest activity of the enzyme was at P_H 5, and gradually diminished on each side of this point. At the extreme alkaline and acid sides a maceration was evident with the enzyme absent. This action is probably due to direct maceration, since most of the extracts had a P_H of about 6.

It was thought that possibly the enzyme was secreted into the surrounding medium, and hence was not detectable in the mycelium. An apple well rotted by *Sclerotinia cinerea* was ground up, extracted with water, and tested for macerating principle, on both apple and potato tissue. No macerating action was detected up to 9 days. The medium upon which the spores had grown was also tested against potatoes, and again the results were negative.

As a last resort various strains of potatoes, of a wide range of mealiness, were used as test tissue for protopectinase activity, with the idea that the most mealy might have middle lamellae most easily hydrolyzed by the enzyme. The extract was made from dried ground mycelia in the usual manner from a young 24-hour growth of mycelia, and maceration tested on strips of potato from each variety. Also a cooking test was made to grade the mealiness of the potatoes. The results on all the potatoes were negative.

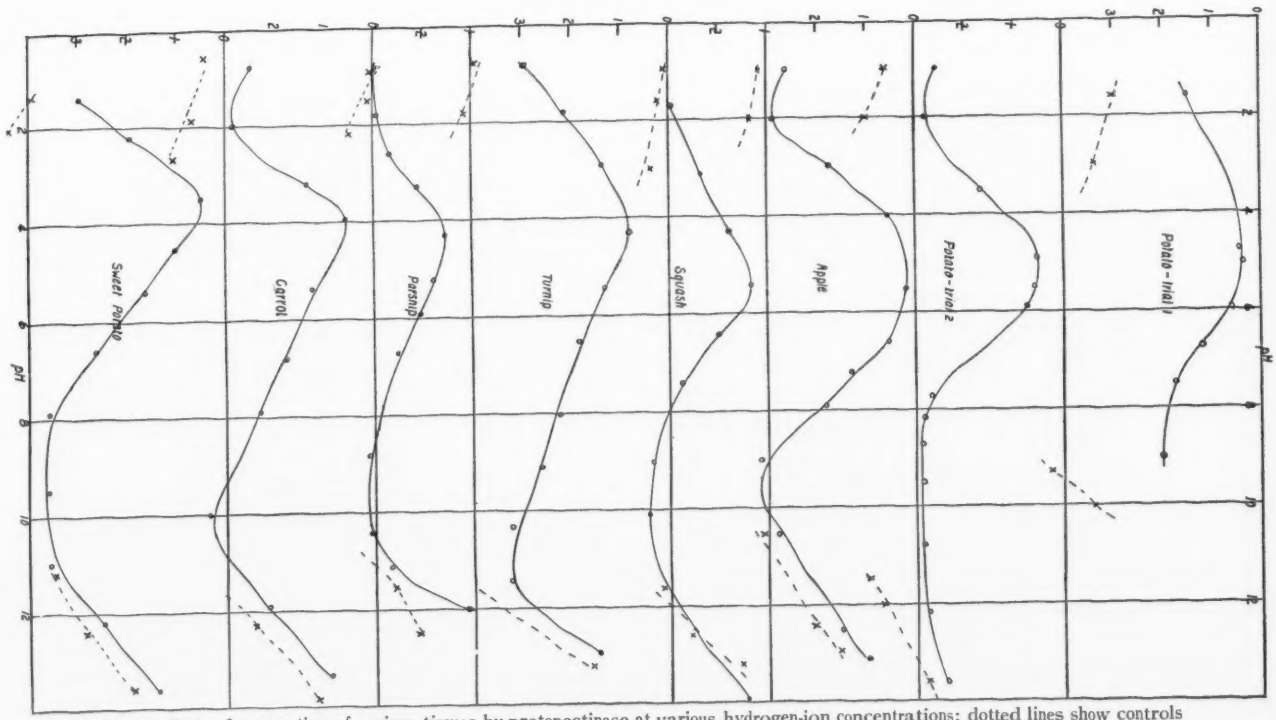


FIG. 1.—Rate of maceration of various tissues by protopectinase at various hydrogen-ion concentrations; dotted lines show controls

The only possible conclusion that could be drawn from the results with *Sclerotinia cinerea* is that there is no intra- nor extra-cellular protopectinase present in the strains studied.

Conditions for production and activity

After the failures already recorded to detect any protopectinase in *Sclerotinia cinerea*, this fungus was abandoned in favor of *Rhizopus tritici* for further study of the properties of the enzyme. A series of experiments was undertaken to determine the factors involved in its production and activity.

A careful study was made of various media to discover which would support a rapid and luxuriant growth of *Rhizopus tritici*. A liquid medium seemed more desirable because of its ease in preparing and handling, and also because of the possibility of testing for the extra-cellular enzyme.

Harter and Weimer (17) found that a decoction of sweet potato was very good for growing *Rhizopus tritici*. Decoctions of sweet potato, white potato, prune, and carrot were made and tested for their ability to support the growth of this fungus. The medium which proved the most efficient and practical was a decoction of carrots prepared by the following formula: To peeled carrots add double the weight of water, steam 4 hours, then filter through gauze and finally filter paper; add 25 cc. of this filtered carrot decoction to 250 cc. Erlenmeyer flasks, plug with cotton, and autoclave for 20 minutes at 15 pounds pressure. The resulting solution is clear and is found to be a very satisfactory medium for growing *R. tritici*.

Peach and prune media were made by soaking the dried fruit over night, then stewing for 2 hours. The juice was then pressed out, filtered, and diluted to the desired strength. Carrot juice supported the best growth, and was followed by sweet potato, white potato, prune, and peach juice respectively.

The acidity of the medium on which *Rhizopus tritici* grows does not affect the protopectinase production to any great extent (20). Jones found that an acid medium was more favorable for protopectinase production by *Bacillus carotovorus*. For spore production a solid medium was preferred. Soaked peach mash, potato agar, prune agar, sweet potato agar, and mashed carrot were all given a thorough

trial. Mashed carrots and potato agar produced a fine growth of spores and in a very short time; then in order of decreasing sporulation came prune agar, mashed potatoes, and mashed peach.

JONES found that the amount of enzyme in the medium surrounding *Bacillus carotovorus* increases with age to 19 days or longer. HARTER and WEIMER (**17**) found with *Rhizopus tritici* that the maximum enzyme content of the hyphae and of the medium is attained in about 24 and 48 hour old cultures. The hyphae were the most active, but the solution or medium upon which the fungus grew increased in activity up to 7 days or more.

TABLE IV

EFFECT OF METHOD OF GRINDING ON PROTOPECTINASE ACTIVITY

No.	SAND (GM.)	TIME OF GRINDING (MINUTES)	TIME FOR MACERATION (MINUTES)
1	0.5	1	53
2	0.5	2	63
3	0.5	3	68
4	0.5	5	69
5	0.5	10	.....
6	0	1	47
7	0	2	52
8	0	3	57
9	0	5	59
10	0	Shaking	68

Since enzymes are sometimes injured by too severe grinding, a comparison was made of the rate of maceration by mycelial hyphae ground in sand, and ground without sand. Table IV shows the results. In each case the concentration was 0.5 gm. of mycelial powder, 25 cc. water, and 0.5 gm. of sand when the latter was used. The results show that enzyme activity is decreased in both cases with the time of grinding. Grinding without sand produced a stronger macerating extract than grinding with sand, as the sand seemed to have a destructive action on the enzyme in every case. One minute of grinding without sand seemed to be the best way to obtain the enzyme extract, and this procedure was adopted. Filtering this extract through paper reduced its activity noticeably; therefore centrifuging was employed.

The time required to extract the enzyme from the ground myceli-

um was ascertained by testing the macerating power of the solution after standing for various periods of time in contact with the ground mycelium, using as before 0.5 gm. of the latter to 5 cc. of water, and grinding for one minute with toluene present. The extract was centrifuged and the macerating activity determined quantitatively on strips of potato, using the drop-weight method already described. Fig. 2 gives the results. The greatest strength of the extract was obtained in 4 days, but an extraction of 10–12 hours is shown by the curve to be the best for economy of time and consistency of results.

Results showed that after the extraction of the powder, centrifuging, and storing with toluene, the extract became weaker very gradu-

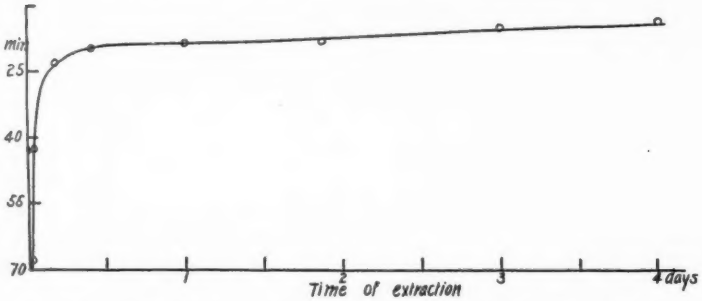


FIG. 2.—Relation between time of extraction of ground mycelium and protopectinase activity of extract.

ally; the activity decreased one-half or more in strength in 3 weeks' time. The dried and ground mycelium, kept at room temperature over concentrated sulphuric acid in a vacuum, lost about half of its activity in 5 months.

An enzyme extract was made as before, using 0.5 gm. of powder to 25 cc. of water. Successive maceration was carried out on strips of potato tissue, the strips being as nearly alike as could be made. Table V gives the results for three different periods of extraction of the mycelial powder. The first five macerations are very similar but noticeably decreasing in strength, until at the eighth maceration the activity is nearly gone. In short time extraction the strength is a function of the time of extraction.

Since every enzyme has a more or less definite optimum range of hydrogen-ion concentration, it seemed advisable to determine this for protopectinase. A series of solutions of varying hydrogen-ion concentration was prepared, therefore, and a number of different tissues used as testing material. The results are presented in fig. 1. The curves of the speed of maceration charted as the ordinates and the P_H as the abscissae show a slight decrease in maceration from P_H 1 to about 2, then a steady increase in maceration up to approximately P_H 5, followed by a corresponding regular decrease to from P_H 8 to 11, depending on the tissue used. Control determinations

TABLE V

EFFECT OF SUCCESSIVE MACERATIONS ON STRENGTH OF PROTOPECTINASE SOLUTIONS

TRIAL	MACERATION TIME (MINUTES)	TRIAL	MACERATION TIME (MINUTES)
Extracted 24 hours		Extracted 1 hour	
1	21.5	1	28
2	22	2	57
3	22		
4	26	Extracted 15 minutes	
5	26		
6	28	1	60
7	36	2	268
8	215		
9	.....		

with heated enzyme show a slow maceration at high acidities and high alkalinities, but none in between. Each individual tissue shows its own characteristic curve, but the general shape of the curves is about the same for all tissues used, and indicates a well marked optimum at a P_H 4.5–5.

Toluol was used as an antiseptic. HARTER and WEIMER (17) found it very successful. It had no macerating effect on the common potato, and it did not retard protopectinase activity. The enzyme did not seem to be affected by the presence of the brass weights used in the determination of time of maceration. Light had no noticeable effect on its activity.

EXPERIMENTS WITH STANDARD PROTOPECTINASE

The maceration of tissues in the sense employed here means the separation of the cells from one another without the disintegration of the cells themselves. It is believed that the relative ease with which this maceration may be brought about is of importance in several ways, among which may be mentioned: (1) the relative susceptibility of tissues to invasion by those fungi which depend on protopectinase for penetrating the host tissue; (2) the firmness of various fleshy tissues, such as tubers and roots, after cooking; (3) the mealiness of potatoes; (4) the retting of flax. In making quantitative investigations of any of these phenomena, it would be highly desirable to have at hand a standard and very active preparation of protopectinase to be used as a reagent. In the following paragraphs are given a few preliminary results with the preparation and use of a standard solution of protopectinase, presented largely to show some of its possible uses.

With the foregoing facts at hand concerning the conditions for the production and use of this enzyme from *Rhizopus tritici*, it was possible to prepare a more or less standard solution of it for use as a quantitative reagent. The standard procedure was as follows: 1 kg. of thinly sliced carrots were boiled in 2 l. of water for 4 hours, filtered, and 25 cc. portions placed in 250 cc. Erlenmeyer flasks and sterilized. These were thickly sown with spores and incubated at 20° C. for 24 hours. The mycelial mats were removed from the flasks, rinsed, stirred in a large excess of acetone for 10 minutes, transferred to fresh acetone for 2 minutes and then to ether, after which they were dried by a fan and then over sulphuric acid in vacuum. A standard extract of this mycelium consisted of 0.5 gm. of it ground for 1 minute in 25 cc. of water containing 2 drops of toluene, and then centrifuged.

A series of comparative maceration experiments was run to discover how long it took the standard enzyme to macerate the various tissues. Ten cc. of the extract was placed in a number of tubes in a water bath at 37° C. Uniform strips of different tissue were cut and the time to macerate them estimated carefully, each determination being run in duplicate. Control strips in water did not macerate. Table VI shows the results of this experiment. A large range in time

of maceration is noted, varying from 2 minutes in pumpkin to 80 minutes in squash. Time was not available for studying the effect of maturity of tissue, position in plant, and other factors which suggest themselves.

TABLE VI

RELATIVE RATE OF MACERATION OF VARIOUS TISSUES BY PROTOPECTINASE

TISSUE	TIME OF MACERATION (MINUTES)
Pumpkin	2, 2, 2
Sweet potato	34, 35
Carrot	41, 42
Potato	35, 34
Rutabaga	66, 63
Turnip	48, 48
Squash	80, 76

The size of the potato tuber and the region from which the test tissue is taken show a difference in rate of maceration. Table VII shows the data obtained on two varieties of tubers. The larger potatoes have a longer maceration period. The differences between cortex and medulla are irregular.

TABLE VII

EFFECT OF VARIETY, SIZE, AND REGION OF POTATO TUBER ON RATE OF MACERATION

EXPERIMENT	VARIETY	SIZE	SECTION	TIME OF MACERATION (MINUTES)
1	Cobbler	Large	Cortex	37, 42
2		Large	Medulla	35, 35
3		Small	Cortex	23, 27
4		Small	Medulla	28, 30
5	Triumph	Large	Cortex	39, 36
6		Large	Medulla	56, 62
7		Large	Medulla	38, 37
8		Small	Cortex	37, 37

An attempt was made to correlate the mealiness of the potato with its rate of maceration. Tubers of uniform size were chosen, peeled, a piece removed from the cortex for a maceration test, and the remainder placed in 1000 cc. beakers and 600 cc. of boiling water added. The potato was boiled until done, as shown by testing with a fork. The mealiness was tested as soon as the potato was removed

from the water by breaking it open with a fork and noting its appearance. The extent of sluffing was estimated by the general appearance of the tuber and the amount of potato particles that were lying in the beaker after the cooking test was completed.

Table VIII shows that there was no regular relation between mealiness, sluffing, and time of maceration. It was thought that the rate of maceration might vary with mealiness, but no regular indication of this could be seen.

TABLE VIII

MEALINESS, SLUFFING, AND RATE OF MACERATION OF DIFFERENT VARIETIES OF POTATO

VARIETY	MEALINESS	SLUFFING	RATE OF MACERATION
Cobbler	+++*	+++	28, 32
Cobbler	+++	++	20, 21
Triumph	+++	+	38, 36
Triumph	+++	+	36, 36
Triumph	+++	++	24, 24
Triumph	+++	+++	32, 31
Triumph	+	+++	25, 27
Triumph	+	+++	25, 26
Triumph	++	+	27, 29
Triumph	+++	+	25, 29
Cobbler	+++	++	30, 31

* +++ very mealy; +++ great deal of sluffing.

MACERATING POWER OF OXALATES

The question as to the macerating ability of oxalates or oxalic acid has been much discussed, and there is little agreement. COOLEY (12) found it present in rotted peaches and plums, but was doubtful whether it was a middle lamella solvent. VALLEAU showed that oxalic acid would soften plum and peach but not potato tissue. SMITH (33) compared the mycelial extract of *Botrytis* with weak solutions of oxalic acid on stems of lettuce, and found that the oxalic acid alone induced a macerating action on the tissue similar to that of the extract of the fungus material, and on the basis of these results came to the conclusion that the dissolution of cell walls noted is due to the oxalic acid secreted by the fungus.

Several common oxalates likely to be found in plants were chosen and used at a concentration normally possible in the tissues. The P_H's were adjusted as shown in table IX, and the maceration noted.

A blank series without oxalate was also run. The plant tissues selected were the potato, apple, lemon, and carrot. Table IX shows that sodium, potassium, and ammonium oxalate have little if any effect on the tissues used. The control shows that an alkalinity of 10 to 11 had a slight macerating effect, while a P_H as low as 1.55 had no effect. Apple tissue seemed slightly macerated by the oxalates, but this effect may be only a slight hydrolysis or softening due to water present.

TABLE IX

MACERATION OF TISSUES BY OXALATES

P_H	POTATO	APPLE	LEMON	CARROT	
2.80	— — —	?	— — —	— — —	0.25 per cent ammonium oxalate
4.80	— — —	— — —	— — —	— — —	
5.52	— — —	— — —	— — —	— — —	
6.46	— — —	— — —	— — —	— — —	
8.58	— — —	— — —	— — —	— — —	
2.10	— — —	— — —	— — —	— — —	0.30 per cent potassium oxalate
4.50	— — —	— — —	— — —	— — —	
4.90	— — —	— — —	— — —	— — —	
6.80	— — —	— — —	— — —	— — —	
9.58	— — —	— — —	— — —	— — —	
1.65	— — —	— — —	— — —	— — —	0.25 per cent sodium oxalate
3.65	— — —	— — —	— — —	— — —	
5.35	— — —	— — —	— — —	— — —	
7.15	— — —	— — —	— — —	— — —	
10.02	— — —	— — —	— — —	Slight	
1.55	— — —	— — —	— — —	— — —	Blank
3.68	— — —	— — —	— — —	— — —	
7.14	— — —	— — —	— — —	— — —	
10.20	— — —	— — —	— — —	— — —	
11.00	— — —	— — —	— — —	Slight	

IV. Pectase

SOURCE AND PREPARATION

This enzyme, which coagulates soluble pectin to a gel, was discovered by FREMY (16) in 1840. He prepared it by the alcoholic precipitation of the juices of several plants.

No thorough search was made for this enzyme. Table I shows the substances which were tested and the results obtained. Corn pollen, clover leaves, and *Sclerotinia cinerea* were the only materials which contained the enzyme.

In the following studies the juice of clover and an extract of corn pollen proved abundant sources for pectase. This was prepared from the clover by first pressing out the juice in a hydraulic press, centrifuging, adding a little chloroform, and setting it away from the light. After 24 hours a coagulum formed which could be filtered. The filtrate was dried by an electric fan, then over sulphuric acid in a vacuum. The dried powder was ground and screened.

Corn pollen was extracted with water for several hours, then centrifuged and was ready for use. A powder of very strong enzyme activity was prepared from this pollen extract by precipitating with two volumes of alcohol, filtering, drying, and powdering.

When *Sclerotinia cinerea* grew on prune juice it secreted pectase, which was indicated by the formation of a flocculent gel in the medium below the mycelium. The enzyme could be precipitated by alcohol from the prune juice in which the fungus had grown.

DETERMINATION

It was found very difficult to develop a quantitative method for pectase estimation, but a qualitative method is relatively simple. Twenty cc. of a 1 per cent solution of lemon pectin[4] is placed in a 50 cc. Erlenmeyer flask, a pinch of $CaCO_3$ added, and mixed well. The determination is carried out at room temperature. An extract of the material to be tested is added to the flask and the time of coagulation and general consistency of the gel noted. The time of appearance of syneresis is also noted, and the general relation between time and consistency used in judging the relative pectase activity. The burden of the determination is left to the individual judgment, which makes it very approximate quantitatively, but easy to detect qualitatively.

CONDITIONS FOR PRODUCTION AND ACTIVITY

Very little has been done with regard to the conditions best suited for pectase activity. It is known that calcium, barium, or strontium must be present in the solution for gelation to occur (**25**), and EULER and SVANBERG (**15**) determined its optimum P_H to be at about 4.3.

[4] The pectin was kindly furnished by Mr. C. P. WILSON of the California Fruit Growers Exchange.

The method used for studying the effect of the hydrogen-ion concentration on pectase activity was as follows. Twenty cc. of 1 per cent lemon pectin was placed in a series of 50 cc. Erlenmeyer flasks and acid and alkali added to bring about the desired reaction. Then 10 cc. of active pectase was added and the P_H determined at once by the potentiometric method. Any change in volume due to adding an acid or alkali was corrected for. A control was run in the same man-

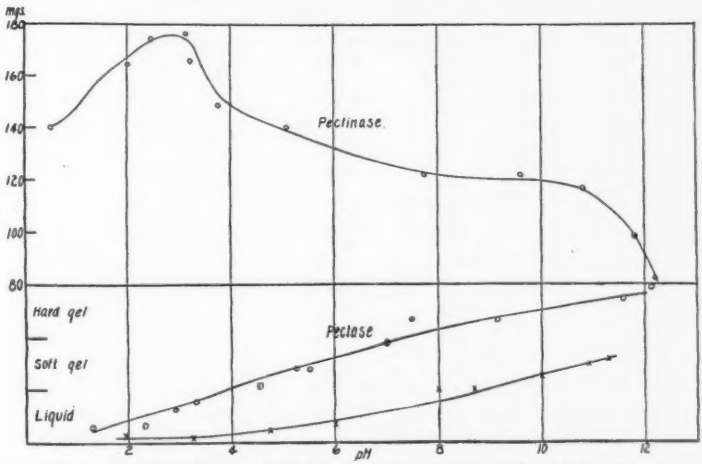


FIG. 3.—Relation between hydrogen-ion concentration and activity of pectinase and pectase; crosses show controls.

ner as the original, using a 1 per cent pectin solution at the different P_H's, but with no pectase present. Fig. 3 shows the results. There was a gradual increase in viscosity as the acidity decreased. Production of a firm gel began at a P_H of 7. In the control a soft gel begins to form at this point. From these data no optimum point or even zone can be detected. From the experiment it is clearly shown that alkalinity alone will produce a soft gel, beginning at about P_H 8, but when combined with pectase produces a firm gel. On the extreme acid side no pectase activity is apparent.

All observations indicate that pectase is very stable. No change in the activity of the dried precipitates was detectable up to 4 months. The extracts were not kept so long, due to contamination,

but were very active until contaminated. The pollen grain powder which had been stored for 6–8 years was still very active. Other pectic enzymes may have been present at one time, but pectase was the only one present after this period.

Filtering through paper did not remove any of the enzyme, but centrifuging was finally adopted, for uniformity with the treatment used for the other pectic enzymes.

V. Pectinase

Sources and preparation

Table I shows the results of running pectinase tests on the same series of materials as used for the other pectic enzymes. Ptyalin, amylopsin, and several of the other enzymes from animal origin were tested, but no pectic enzyme was found, and hence in a way they acted as a control on the methods used.

The best sources of pectinase were *Rhizopus tritici*, *Sclerotinia cinerea*, and *Botrytis cinerea*. In all cases the enzyme could be prepared by grinding the material with water for several hours, then precipitating the filtered extract with alcohol.

A mycelial powder was made by inoculating sterile flasks of prune juice or carrot decoction with *Sclerotinia cinerea* and *Rhizopus tritici* respectively, and incubating at 21° C. for 24–48 hours, then harvesting, washing, and drying by Dox's method. From this dried powder a standard extract was made by mixing 0.5 gm. of the powder with 25 cc. of H_2O, and leaving for 10–14 hours. After centrifuging, the extract was ready for use. The medium upon which the *Rhizopus* had grown for several days was also a good source of pectinase. It may be used as it occurs in the liquid medium, or precipitated with two volumes of alcohol.

Determination

A pectinase determination is carried out by estimating the reducing substances formed by the action of the enzyme on pure pectin. Pectinase hydrolyzes soluble pectin, and also the gel produced by the action of pectase on pectin, to reducing substances, probably arabinose and galactose (**14**). To 20 cc. of 3 per cent pectin is added 10 cc. of enzyme extract, made acid with HCl, and placed in an incubator at

40° C. for 24 hours. A control is made by omitting the pectin. The pectin used was found to have no reducing action. After hydrolizing the solution was clarified by one of the common methods, and reducing sugars run by the picric acid method (41). By correcting for the control and calculating the reducing substances as galactose, an accurate but comparative indication of pectinase content was obtained.

Conditions for production and activity

The fungi in question produced pectinase on the same media on which they produced the other enzymes. The age of the culture for maximum pectinase production was found to be somewhat older than that found for maximum protopectinase activity. A 48-hour culture was used, but there were indications that an older culture would produce a stronger pectinase reaction.

Pectinase was found to be very stable, judging from observations made on the activity of extracts kept for months under toluene. The dried alcoholic precipitations of pectinase were also rather stable, as well as the dried powdered mycelium when kept over concentrated sulphuric acid in a vacuum. The activity of pectinase was somewhat impaired by filtering, so centrifuging was strictly adhered to.

The concentration of pectin on which the pectinase extract acted had considerable effect on the amount of reducing substances formed. Solutions of pectin of 0.5–5 per cent concentration and enzyme extract were mixed together and set aside for 24 hours, and reducing activity was determined. The results were irregular, but indicated in general the same fact as for most enzymes, that increase in concentration of substrate increases the work done per unit of enzyme, but the relation is not linear. The data are presented in fig. 4.

VI. Existence of three pectic enzymes

As was stated in the introduction, some writers assume but two pectic enzymes, on the basis that the same enzyme causes both a maceration of plant tissue and a hydrolysis of soluble pectin. One of the main objects of the present study was to secure decisive information on this question. In this section are collected the main facts at hand bearing on the identity of these enzymes, including not only the facts previously discussed, but also evidence adduced to show

that there are three separate enzymes. For the sake of clarity a scheme is presented in table X which shows the composition of the pectic substances, so far as this is now generally accepted, and the relations of three possible enzymes to these substances. The facts that bear on the identity of the pectic enzymes, and which substantiate the existence of three of them follow.

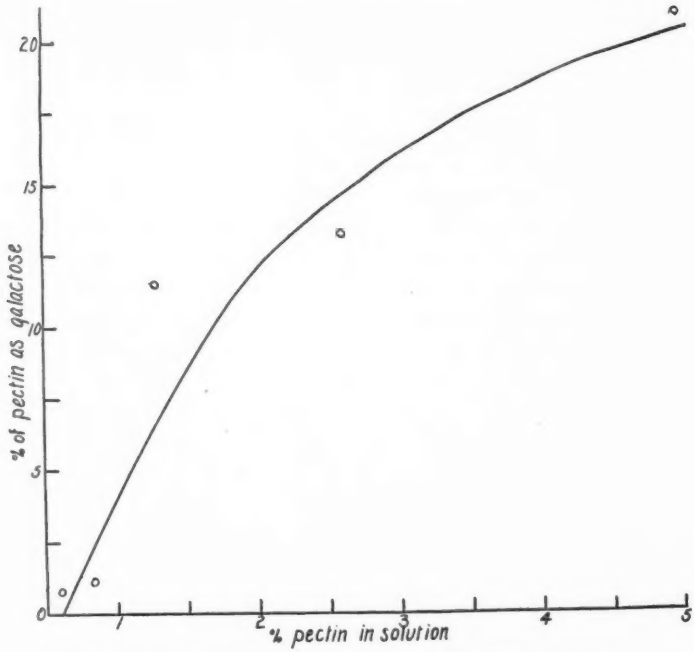


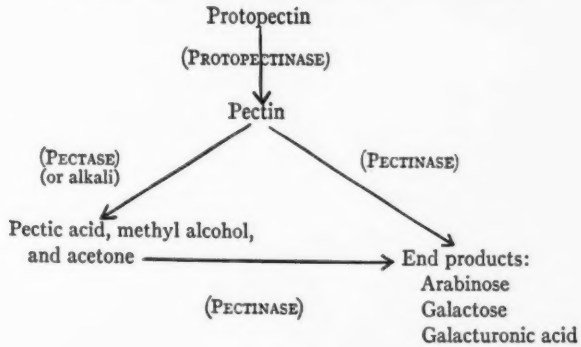
FIG. 4.—Relation between pectinase activity and amount of substrate present

One and possibly the only method for the identification of enzymes is by the nature of their end products, and the changes they produce. Table X gives the relation of the pectic enzymes to one another and to their end products. TUTIN (**35**), studying the effect of alkalies and of pectase on pectin, found methyl alcohol, acetone, and a salt of pectic acid as the decomposition products. He concludes that pectin is probably the dimethylisopropenyl ester of pectic

acid. TUTIN does not believe that pectin exists in the plant as pectose or protopectin. This CARRÉ (**11**) confutes, claiming on the basis of chemical and microscopic evidence the presence of an insoluble pectic constituent in apple tissue called pectose, which is converted by hydrolysis to pectin. CARRÉ believes that the long extraction carried out by TUTIN caused hydrolysis.

TABLE X

RELATIONS AMONG PECTIC SUBSTANCES AND THEIR ENZYMES



The writers repeated the work of TUTIN. A 1 per cent solution of lemon pectin was treated in a cold aqueous solution with 13.4 per cent of its weight of NaOH (the amount neutralized by pectin in the cold), made slightly acid, and fractionally distilled. The SCUDDER and the SCUDDER and BRIGGS tests (**32**) were used for methyl alcohol, while the LIEBEN iodoform test (**38**) was used for acetone. A test for both methyl alcohol and acetone was obtained with the methods used. This corroboration of TUTIN's results indicates that pectase must be an esterase; and since there is no evidence that ester linkages are involved in any of the other pectin hydrolyses, pectase must be different from protopectinase and pectinase. The specific nature of the maceration action on tissue and of the production of sugars from pectin makes the last two enzymes appear quite distinct.

Since the differences in temperature of inactivation may form a basis of separating enzymes, and hence of proving a lack of identity among them, this point was determined for the three enzymes under

discussion. Each was prepared according to the methods outlined, and then the enzyme solutions in test tubes were exposed for 20 minutes to various temperatures. They were then cooled, and the

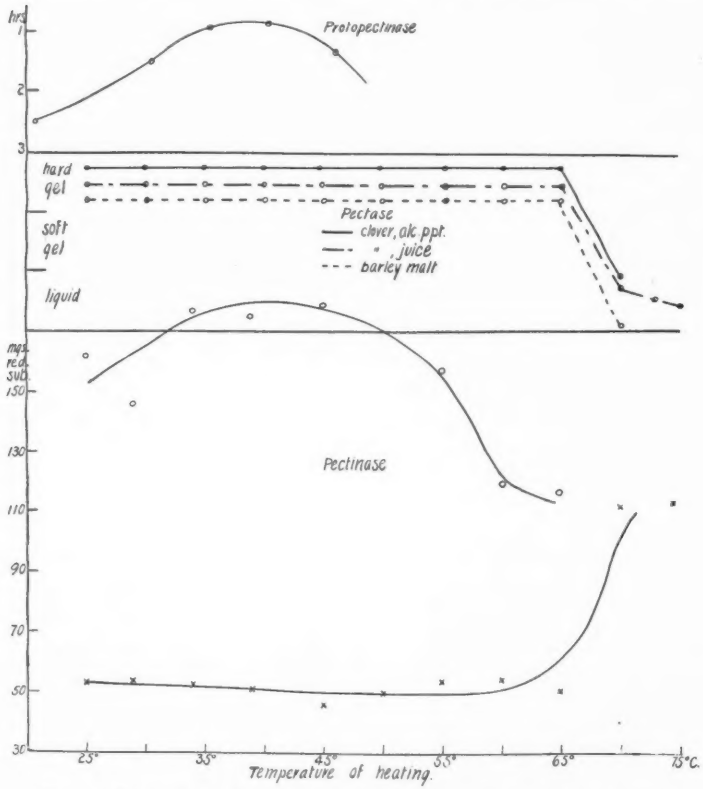


FIG. 5.—Temperature of inactivation of pectic enzymes; crosses show control

activity measured at 20° C. in the usual way. The results are shown in fig. 5.

It will be seen that the temperature of inactivation of protopectinase is rather sharp at about 48° C., that of pectinase rather vague at about 60° C., and that of pectase at about 68°–70° C. The first figure agrees fairly well with that of 51° C. found by JONES (**24**).

This variation of temperature in inactivating the enzyme made it possible to use this as a method of separation and identification. This was performed successfully in the case of *Rhizopus tritici* extract, which contains protopectinase and pectinase. Again, the three enzymes were mixed and their activities checked successively by heating the enzymes to their thermal death points, first eliminating the protopectinase, then the pectinase, and finally the pectase.

The P_H of a medium in which an enzyme acts has much to do with the rate of its activity. Figs. 1 and 3 show the effect of the hydrogen-ion concentration on the activity of these enzymes. The optimum reaction for protopectinase is P_H 5, and of pectinase 3; that of pectase is indefinite, but is greater than 7. These differences in optimum reaction are significant and appreciable, and should be of service in distinguishing the three enzymes.

TABLE XI

FRACTIONAL ALCOHOLIC PRECIPITATION OF MIXTURE OF THE THREE ENZYMES

No. of fraction	Alcohol (per cent)	Precipitation	Protopectinase	Pectinase	Pectase
1	25	Medium	++++	−	−
2	50	Light	++	+	−
3	85	Medium	++	++	−
4	90	Light	+	+++	−
5	95–98	Light	+	−	+++

The sources of the various enzymes have been studied to some extent. Table I gives a list of materials tested for pectic enzymes in the present work. In no case so far have all three enzymes been found together. It would be futile to attempt an explanation of this at present. It is a strong argument, however, in favor of the non-identity of the three enzymes.

Alcoholic precipitation was attempted in the separation of the pectic enzymes. A fractional precipitation was tried on a mixture of *Rhizopus tritici* extract and clover juice. The method adopted was to add alcohol until a precipitate was noticed, filter after 3 hours of standing, and then increase the percentage of alcohol until another precipitate was recognized. Table XI gives the results. It will be seen that protopectinase activity decreases with the percentage of alcohol used, and with successive precipitates. At the same time the

pectinase and pectase activities increased. These results also favor the view that there are three pectic enzymes.

A study was made of the adsorption of these enzymes under varying conditions on various adsorption reagents, as kaolin, charcoal, fullers' earth, and aluminum hydroxide. LEVENE and WEBER (27) used this method for the purification of enzymes with some success. HEDIN (23) also had success by the differential adsorption of enzymes on charcoal and kieselguhr, the charcoal removing beta-protease, and the kieselguhr alpha-protease.

Adsorption of protopectinase on Lloyd's reagent (hydrated aluminum silicate) was attempted. Thirty gm. of the reagent was added to 1000 cc. of carrot medium upon which *Rhizopus tritici* had grown for two days, and the mixture shaken every few hours. The reagent removed protopectinase from the solution quantitatively. The reagent with the adsorbed enzyme was then placed in a series of solutions of varying P_H, using 3, 6, 7, 8, and 12, in the attempt to free the enzyme again, but unsuccessfully. Kaolin was next used as an adsorption agent. A 3 per cent mixture of kaolin and protopectinase extract was made and left for 14 hours with occasional shaking. The kaolin removed the enzyme from solution, but again treatment of the adsorption complex with a range of solutions of P_H 3–9 failed to remove the enzyme. Norit was also tried in the same concentration and manner of treatment as kaolin, and negative results were again obtained.

The pectinase used in these experiments was prepared from mycelia of *Sclerotinia cinerea* and *Rhizopus tritici*, and from the carrot medium upon which the latter had grown. Charcoal (norit), Lloyd's reagent, and kaolin were used to adsorb the enzyme from each of the solutions, in the following manner. Thirty gm. of the adsorption reagent was added to 1000 cc. of the enzyme extract or solution upon which *Rhizopus tritici* had grown for 2 days. The mixture was shaken occasionally during a period of 15 hours, then centrifuged, and the solid dried by a fan and then over sulphuric acid in a vacuum. Lloyd's reagent removed the pectinase quantitatively from the extract, but kaolin and norit were not quite so efficient. The enzyme could not be freed from these materials by digesting in solutions, the P_H's of which varied from 4 to 9.

A strong pectase was prepared by extracting corn pollen with water for 10 hours and centrifuging the liquid. Kaolin, Lloyd's reagent, and norit were again used for adsorbing the enzyme. The first two reagents removed the enzyme completely, but the norit only incompletely. By varying the P_H between a range of 4 to 9, no pectase activity could be recovered from these adsorption complexes. From the Lloyd's reagent a slight activity was recovered at P_H 11.6, but as the control showed also a slight gelling, the enzyme action is doubtful. Table XII shows the results of these adsorption studies in a more collected form. They were not successful in showing any differences among the three enzymes.

TABLE XII

ADSORPTION OF PECTIC ENZYMES

ENZYME	ADSORPTION REAGENT	ADSORPTION	LIBERATION
Protopectinase	Lloyd's reagent	Complete	None
	Kaolin	Complete	None
	Norit	Complete	None
Pectinase	Lloyd's reagent	Incomplete	None
	Kaolin	Incomplete	None
	Norit	Incomplete	None

It was noticed that the protopectinase activity varied with the method used, whether the enzymes were removed from the débris of cells by filtering or centrifuging. For that reason the effect of filtering and centrifuging was carefully tried out on the three pectic enzymes. Table XIII gives the results of the experiment, which might be summed up by saying that filtering decreases only protopectinase activity. Nevertheless centrifuging was adopted for all three enzymes.

Practically all of these criteria concerning the existence of these pectic enzymes indicated that there are three. From what information is available now concerning the composition of the pectins, and the occurrence and function of these enzymes, the following generalizations may be offered. Protopectinase macerates the tissues, forming pectin, thus making it possible for the mycelia of the fungi to penetrate that tissue. Then pectinase acts on the pectin and hydrolyzes it to the simpler end products, and these serve as food for the

fungi. Pectase coagulates soluble pectin. What its usefulness may be is not known at present. WILLAMAN (40) suggested that in the case of *Sclerotinia cinerea* the gel formed conveys hydrophylic prop-

TABLE XIII

EFFECT OF FILTERING AND CENTRIFUGING ON ENZYMES

ENZYME	SUBSTRATE	ENZYME TREATMENT	RESULTS
Pectase	10 cc. pectin, 1 per cent $CaCO_3$.....	Centrifuged First filtration Second filtration Third filtration Fourth filtration	Hard gel
Protopectinase	Potato tissue.....	Centrifuged First filtration	Maceration time 18 minutes Maceration time 25 minutes
Pectinase	10 cc. of 3 per cent pectin.....	Centrifuged First filtration Second filtration Third filtration Fourth filtration	Mg. reducing substance as glucose 498 501 510 525 515

erties on the plum tissues that are advantageous to the fungus in the "mummy" stage of the parasitism. Some of the properties of the pectic enzymes are summarized in table XIV, so that the factors discussed can more easily be compared.

TABLE XIV

SUMMARY OF PROPERTIES OF PECTIC ENZYMES

	PROTOPECTINASE	PECTASE	PECTINASE
Substrate.....	Protopectin	Pectin	Pectin and pectic acid
Gross effect of action.....	Maceration of plant tissue	Coagulation	Hydrolysis
Type of linkage attacked..	Unknown	Ester	Ether (glucosidic)
Point of thermal inactivation.....	48° C.	68°–70° C.	60° C.
Optimum P_H.....	5	Above 7	3–6

Summary and conclusions

1. Protopectinase (pectosinase) has been prepared from *Rhizopus tritici* and *Bacillus carotovorus;* in the former both the intracellular and extracellular, and in the latter only the extracellular form was used.

2. Pectase has been prepared from clover leaf sap, corn pollen, and the mycelium of *Sclerotinia cinerea*.

3. Pectinase has been prepared from the mycelia of *Rhizopus tritici*, *Sclerotinia cinerea*, and *Botrytis cinerea*, and from barley malt.

4. Quantitative methods for measuring the activities of these enzymes have been developed, and the conditions for their production and action determined.

5. A careful search among many cultures of *Sclerotinia* and related forms failed to disclose protopectinase activity in any of them.

6. The rate of maceration of several tissues, including large and small potato tubers, different regions of the tubers, carrot, squash, sweet potato, pumpkin, turnip, and apple was measured.

7. Convincing evidence was obtained in support of the view that there are at least three distinct pectic enzymes, namely, protopectinase, pectase, and pectinase. The lines of evidence are (1) all three enzymes have not been found in the same plant material; (2) they have quite different temperatures of inactivation; (3) they differ somewhat in their optimum P_H; (4) they can be separated more or less completely by alcoholic precipitation; (5) protopectinase is appreciably adsorbed onto filter paper, while the others are not; (6) pectase probably hydrolyzes ester linkages, while the others hydrolyze other linkages.

8. Sodium, potassium, and ammonium oxalates were found not to macerate tissues of lemon, potato, carrot, and apple, over a wide range of hydrogen-ion concentration.

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[*Accepted for publication January 3, 1927*]

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ALTERNATION OF GENERATIONS IN RELATION TO REDUCTION DIVISION

NILS SVEDELIUS

The problem of the alternation of generations of late has come once more to the front in botanical discussions. This probably is due to the fact that our knowledge of the life history of the lower plants, and especially the cytological side of it, has undergone a very important extension. The significance of reduction division in marking limits of the generations has become a hotly contested question. Opinions differ as to whether the alternation of generations established by HOFMEISTER (22) really has any connection with the alternation of haploid and diploid states of the nuclei. A survey of the various opinions that have been expressed on this question, and of the terminology used by the disputants, leads one to agree with the pronouncement of BRAUN (3), made as far back as 1875, that "generation is really an elastic conception."

The introduction of the cytological element into the problem of alternation of generations, by STRASBURGER (40), gradually but consistently brought us to the point where the concept "antithetic alternation of generations" became synonymous with alternation of haploid and diploid states of the nuclei. The reaction against this conception from BUDER (4), KYLIN (30), and RENNER (36), who regard generations from a purely morphological point of view, has led to the surrender of the antithetical conception of the alternation of generations, with the result that the life cycle of a plant can be divided into any number of generations. Consequently, one still finds in many modern works on the alternation of generations the conception which was current as early as 1860. The explanation of this, of course, is the difficulty, not to say impossibility, of fixing the meaning of the term "generation." The adherents of the purely morphological view find their support in a certain principle of priority, since all cytological aspects of the problem were beyond the purview of HOFMEISTER; indeed, they were at that time unknown. On the contrary, a sharp line between external and internal morphology (anatomy and cytology) cannot easily be drawn; therefore

the attempt to characterize the generations by cytological marks cannot be stamped as incorrect (BUDER 4), because the generations were originally distinguished by purely morphological characters. Laws of priority are impossible here, they may be employed in giving plants and animals scientific names, but the history of science shows that the principle of priority cannot hold good in the sphere of general terminology. Language is more conservative than thought, and words live on and continue to be used though their meaning may have changed more than once. We have only to think of the concepts "cell," "fertilization," "phanerogam," "cryptogam," etc. Our modern conception of cell is far removed from that which ROBERT HOOKE denoted by this name in 1667; that is, we still use the word but put a different meaning into it.

The idea of "fertilization" cannot be explained nowadays without reference to the cytological conditions, although the conception "foecundatio" with reference to plants is met with as early as in the works of CAMERARIUS, KOELREUTER, and LINNAEUS. What they meant, however, was probably about the same as we now mean by "pollination." The word fertilization, of course, has never been altered, but the conception now indicated by this term includes much more than the mere transference of pollen grains to the stigma. The same thing has happened to the expression "alternation of generations." In the course of time this, too, has been very considerably changed in meaning. HOFMEISTER'S (22) conception is a very different thing from what CHAMISSO and STEENSTRUP (39) had much earlier called by this name, on account of their discoveries concerning the evolution of the Salpae, Medusae, and Trematodes. Now, however, instead of first insisting upon a definite conception of alternation of generations, it is held to be more important to show whether there exists a universal and common explanation of, or a biological connection between the different phenomena which have, by various authors at different times, been designated as "antithetic" and "homologous," or "morphological" and "cytological" alternation of generations. Only after such an investigation can we gain a clue to the explanation of the remarkable phenomenon which recurs in widely separated groups of plants, in each of which the life cycle can be divided into rhythmically recurring phases or sections

that sometimes become separate individuals. These phases are sometimes somewhat alike; on the other hand they sometimes differ considerably in degree of development; in other words, I mean that singular relation of alternation in the ontogenetic development of plants which NÄGELI (**32**) called "one of the most remarkable phenomena of the plant-world."

Could all these types of alternation be shown to be variants of the same phenomenon, then the solution of the much contested problem of alternation of generations might be said to have been begun. To this solution I shall now endeavor to make a contribution.

It is characteristic of the first observers of the alternation of generations in both animals and plants that, as a rule, they make no attempt to explain this phenomenon. CHAMISSO, STEENSTRUP, and HOFMEISTER all content themselves merely with establishing the fact itself. The discoveries were themselves of such a sensational kind that the establishment of the fact itself sufficed and left no need of the addition of an explanation, which perchance could not then have been given. ČELAKOVSKÝ (**6**) states:

> It is a perceptible fault of most presentations of the alternation of generations that the actual phenomena are simply registered without any effort being made to explain the alternation of generations, so that it remains an astonishing but incomprehensible fact.

Very soon, however, attempts at explanation were forthcoming. For instance, the zoologist LEUCKART thinks that the alternation of generations is a sort of division of labor between food preparation and reproduction. Something of this kind may perhaps have been in the thought of HOFMEISTER (**22**), when he remarked: "Vegetative life in mosses is exclusively of the first generation, fruit formation of the second. In ferns the case is almost the reverse."

The problem of the significance of alternation of generations is intimately connected with that of the origin of the generations; for, generally speaking, if a biological explanation of the significance or work of any particular organization is sought, it is necessary to know how this organization arose. In other words, a knowledge of its phylogenetic origin is the first condition for discovering the biological significance of any organization.

The first attempts at explanation of the alternation of generations were altogether on the lines of the Darwinian theory of evolution. The alternation of generations was regarded as being similar to the metamorphosis of the individual organ. The first generation, the "protophyte" of CĚLAKOVSKÝ (**6**), was followed by the second, the so-called "antiphyte," in the same manner that a higher stage of evolution follows a more primitive one in the development of an individual organ. Such an explanation, however, does not really touch the kernel of the problem.

Other attempts to solve the problem more from the biological side were made for instance by NÄGELI, who thought the alternation of generations was *inter alia* an "adaptation to the alternation of the seasons," so that at the end of a vegetation period another generation arises which is better adapted to the new surroundings. NÄGELI admits, however, that he has not hereby solved the problem. Thus he says at the close:

> This fact of the alternation between the two generations to which the ontogenesis of the main order of the vegetable kingdom is due is one of the most remarkable phenomena of phylogenetic evolution. For the causal explanation of this fact of alternation I am unable to suggest anything but the struggle for differentiation.

To enter into all the numerous attempts at an explanation of the alternation of generation is of course impossible. I shall therefore only examine the most important explanation, namely, the hypothesis offered by BOWER (**1, 2**), which was later adopted by WETTSTEIN. This may be termed a migration theory, because the alternation of generations is looked upon as evolved in connection with the migration of plants to land, whereby the higher orders of plants represent "so many steps of the great process of the adaptation of a series of plants, which were originally purely aquatic, to a terrestrial life" (WETTSTEIN **50**). In the same measure that plants became more and more decidedly terrestrial in habit, the sporophyte became more and more dominant, while the gametophyte, which was better adapted to an aquatic life, suffered reduction. This hypothesis finds its chief support in the circumstance that within the series moss-fern-seed plants the sporophyte proves to be organized more for land life than the gametophyte, the organization of which shows

it to be well adapted to aquatic life, and the fertilization of which by means of spermatozoids is absolutely dependent on water. This migration theory was further combined by BOWER (2) with a purely morphological theory to explain the rise of the sporophytes, the so-called interpolation theory. The sporophyte is here regarded as a new formation, arisen and developed from the zygote, which has gone on its own way of evolution. The sporophyte, as it were, is interpolated between two gametophytic generations, and the considerable difference between the generations is naturally explained by regarding the sporophyte as a new formation adapted to terrestrial life, while the gametophyte still remains a more or less pronounced water plant, which still preserves the peculiarities of organization acquired during its aquatic period. This interpolated organism is supposed by BOWER (2) to proceed in such wise that the sporogenous cells, which were originally the only ones formed from the fertilized ovum or zygote, are transformed by "sterilization" into a vegetative, food making system. Finally these vegetative tissues increase more and more, the sporogenous tissue becoming more and more limited and localized; lastly there appear definite accessory organs of various functions; for instance, leaves for the preparation of food, organs for the exposure of the sporangia, etc. The theory of interpolation and sterilization was then worked out in detail by BOWER (2). His theory gained wide acceptance; yet it must be emphasized that numerous investigators have been critical toward the ideas of interpolation and sterilization, which, in this theory, have come to be regarded as antithetical. PRINGSHEIM (34), by his discovery of apospory in mosses (the production of protonemata directly from the seta), soon after FARLOW's (15) discovery of apogamy in ferns, came to the conclusion that the alternating generations "also possess a homogeneous morphological character," that is, they are homologous. PRINGSHEIM (35) became thereby the founder of the theory of the homologous alternation of generations, which is now opposed to the antithetical theory of CĚLAKOVSKÝ and BOWER. In full agreement with this conclusion, PRINGSHEIM assumed that the sporangia, antheridia, and archegonia (or oogonia) were all homologous. These two theories of alternation, the so-called anti-

thetical and the homologous, have thus been irreconcilably opposed to each other.

The antithetical theory gained increasing support, and the biological explanation of the alternation of generations as a migration theory has still further contributed to its establishment. Still more important aid to this end, however, was furnished by the great cytological discovery that the essential feature of fertilization is a conjugation or fusion of nuclei followed by reduction division. This purely cytological school, whose most eminent representative was STRASBURGER, has still further strengthened the position of the antithetic theory, by showing that the cytological cardinal points, the fusion of nuclei and the reduction division, strictly coincide with the limits of the two generations determined by HOFMEISTER on quite different morphological grounds.

There is of course a great deal in the antithetic migration theory which is undoubtedly attractive. The fitness of the gametophyte for an aquatic life is as undeniable as is that of the sporophyte for terrestrial life. The question arises, however, whether this theory still holds good in view of recent discoveries regarding the history of development of the lower plants, discoveries which have been made since the theory was first propounded. The theory found justification as long as the alternation of generations of higher plants (mosses, ferns, and phanerogams) was the only one known. The discovery of a like alternation of generations in the algae, which have never migrated to land, must arouse suspicions; therefore the origin of the alternation of generations is not explained biologically by the theory of migration from water to land.

The first discovered cases of alternation of generations appeared to support the migration theory, to some extent at least, since in *Dictyota* both generations (except for the number of chromosomes and the reproductive organs) are exactly alike. In this case, also, we had to do with a plant both of whose generations lived under identical external circumstances. This discovery of the alternation of generations in *Dictyota* by WILLIAMS (**51** **52**) and HOYT (**23**) aroused great interest. STRASBURGER (**42**) finds *Dictyota* "remarkable" from the standpoint of the alternation of generations, as the

generations are alike, and says this has now become a problem to whose solution we must apply ourselves. In this connection it may be of interest to mention that when NÄGELI (**32**) in 1884 discussed the alternation of generations, suspected even then, between sexual individuals and tetrasporic individuals in the red algae, he adduced as one of the weightiest arguments against this view the fact that the supposed generations were so similar. The knowledge which had been obtained as to alternation in mosses, ferns, and phanerogams exercised such an influence on opinion regarding the alternation of generations, that NÄGELI declared outright that it was inherent in the nature of generations to be different.

Meanwhile our knowledge of the life history of the lower plants was extended more and more. As early as 1907 YAMANOUCHI (**53**) showed that the reduction division in *Polysiphonia* takes place at the division of the tetraspore mother cell, and that the red algae afford an instance of a regular alternation of generations between a haploid generation with male or female organs, and a diploid, spore forming generation. A noteworthy complication here is the diploid nature of the cystocarp. That is, the morphologically distinct generations do not fully coincide with the phases of development distinguishable by the single and double chromosome numbers. The conditions found in *Polysiphonia*, as later investigations by LEWIS (**31**), SVEDELIUS (**43, 44**), and KYLIN (**26**) have shown, recur in *Griffithsia*, *Delesseria*, *Nitophyllum*, *Rhodomela*, etc., and in all probability in the majority of the Florideae. Meanwhile there arose an interesting question regarding those Florideae which never form tetraspores, for there are many such. The writer (**45**) succeeded in finding in *Scinaia* a type that gave an answer to this question. In this genus the reduction division occurs immediately after fertilization. The same thing occurs, as was shown later by KYLIN (**28, 29**), in other Florideae belonging to the Nemalionales (*Nemalion*, *Batrachospermum*, and probably in all other Nemalionales). How shall these differences in a group which is incontestably uniform phylogenetically be explained? In my opinion (**45–48**) these different types can only be accounted for on the supposition that in the course of their evolution there has been a postponement in the time of occurrence of the reduction division. In the undoubtedly primitive *Scinaia*

type, the reduction division occurs immediately after the nuclear fusion. In other derived forms the reduction division for some reason has been dropped at this point, and thus the whole cystocarp has become diploid. The diploid carpospores thus formed germinate and give rise to diploid plants, which form no sexual organs. Reduction division occurs in their monosporangia, however, so-called tetraspores being formed, at whose germination plants arise which are able to carry out the formation of male and female organs. It is thus, in my opinion, that all at once a diploid generation may arise. I am unable to say what is the cause of this delay of the reduction division. Possibly hormones may play some regulating part; a field of work is here open to the physiologist. An attempt must be made by variation of the external conditions to influence reduction division in such wise that it is either postponed or compelled to occur earlier.

The fact should be emphasized that by the delay of reduction division in the Florideae, in the manner described, a new generation (the tetrasporic plant) arises at one stroke. It has not arisen by a progressive sterilization, as the interpolation theory of BOWER (2) assumes, but suddenly.

This assertion, however, does not exclude the possibility of really interpolated organizations likewise occurring. The Floridean cystocarp, for example, arose, in the writer's opinion, by the later formation of sporogenous filaments from the zygote by "sterilization," whereas the zygote in more primitive forms was merged into spore formation, as still happens in the Bangiaceae. One may be fully justified in speaking in the spirit of BOWER both of interpolation and sterilization. I think it better, however, not to term the Floridean cystocarp a "generation," but detailed discussion of terminology would lead too far in this connection.

Why has the new Floridean plant body which arose so suddenly in their evolution no sex organs? It is probably because the diploid structure, for some reason, hinders this. On the other hand, immediately after the reduction division has occurred, a haploid generation arises which carries on the formation of gametes, etc. The sporophytic generation, which forms no gametes but spores only, is consequently a result of the delay of the reduction division.

From this it follows that this spore forming generation must be regarded as morphologically homologous with the original haploid one. This view approaches the so-called homologous theory of the alternation of generations of which I just now called PRINGSHEIM the founder.

It may be asked whether there are other groups of plants in which the same process may be conceived to have taken place. Yes! For example, the alternation of generations of *Dictyota* is capable of easy explanation in this way, and how, otherwise, can the perfect agreement of the gametophyte and the sporophyte be explained? The little we know of the diatoms seems to indicate that here, also, a similar delay of the reduction division has taken place. The plankton diatoms (*Diatomeae centricae*) are in all probability haploid, with the zygote as the only diploid phase, while the *Diatomeae pennatae* are diploid, the gametes being the only haploid cells. The second type has proceeded from the first by the delay of reduction division.

Might not this hypothesis also be made use of for the Archegoniates and phanerogams? The difficulties here encountered are considerably greater because the gametophyte generation has undergone such a tremendous reduction that hardly anything is left of it.

Meanwhile, it is noticeable that in recent times new views are being asserted respecting the relative degree of evolution of such plants, for example, as the liverworts. I am not a bryologist by profession, but I may here point out that GOEBEL (**18**) in the latest edition of his *Organography* expresses views concerning these plants which differ considerably from those which have been influenced by the so-called interpolation theory. GOEBEL's idea is that a comprehensive reduction has taken place in the series of the liverworts. Types like *Riccia*, with their nearly total devotion of the zygote to spore formation, are now placed not at the beginning but at the end of the evolutionary cycle, this condition being regarded as the result of extensive reduction. The much discussed *Anthoceros* also is regarded by GOEBEL as relatively primitive. It is noteworthy also that the well known stomata of the *Anthoceros* sporogonium are by no means to be regarded as new formations of the sporophyte.

They are indeed found fully developed, even on the gametophytes of other liverworts. Gametophytes and sporophytes, then, display much greater correspondence than was at an earlier date believed possible. On the homologous theory this is perfectly comprehensible. It is just the primitive forms which ought to display the greatest likeness in their two generations.

Another noteworthy feature found in the Pteridophytes is the fact that, in the Eusporangiates, for example, where the development of the sporangia is consummated inward from the original surface cells, the antheridial development on the prothallia likewise proceeds inward; while in the Leptosporangiates the development of both sporangia and antheridia proceeds from within outward. Still more examples of this kind might be quoted. What has been brought forward proves that, at any rate, far greater morphological correspondence prevails between gametophyte and sporophyte than had previously been assumed, and this seems to lend more support to the homologous theory of alternation than to the antithetical interpolation theory. I therefore wish to point out that the antithetical interpolation theory itself is not unassailable, but, on the contrary, must be regarded as being in important particulars no longer in full agreement with well known facts.

What is the bearing, in this respect, of the theory of migration? The fact that the larger groups of the higher plants represent as it were different stages in the migration of the plant world to land, of course, is too clearly manifest to be denied. The question may be raised, however, whether an exactly similar course of development, leading to the complete supplanting of the original gametophyte by an absolutely dominant sporophyte is not present also in organisms which have never migrated to land.

I believe that the Phaeophyceae present a very evident development of this kind. The noteworthy discovery by SAUVAGEAU (**37, 38**) of the alternation of generations in the Laminariaceae has thrown an entirely new light upon the whole life cycle of this group. We now know that the prominent phase of *Laminaria* is the sporophytic phase in an alternation of generations, while the gametophyte is a small, insignificant alga filament formerly perhaps described as a distinct species. Thus even here the sporophyte

generation prevails over the gametophyte to as high a degree as in the Pteridophyte group, although both generations still live in the sea.

Furthermore, this discovery regarding *Laminaria* is illuminating for algae of the *Fucus* type also. Few plants, I suppose, have been the object of such varied interpretation as *Fucus*. Algologists have long noted the anatomical similarities shown by *Fucus* and *Laminaria*. *Fucus*, however, is a sexual plant and *Laminaria* an asexual one. The discovery by STRASBURGER (41), FARMER and WILLIAMS (16), and YAMANOUCHI (54) that reduction division in *Fucus* precedes the formation of eggs and spermatozoids did not make the matter clearer. In accordance with what was known about ferns, it might formerly have been supposed that *Fucus* is haploid, but this was found not to be the case. The life cycle of *Fucus*, therefore, was often quoted with satisfaction by those who opposed the view that the alternation of generations is a nearly universal phenomenon in the plant world, and especially by those who denied the significance of the reduction division for the determination of the limits of the generations.

The discovery of the alternation of generations in the Laminariaceae, however, puts the life history of *Fucus* in another light. Several Laminariaceae, for example, *Saccorhiza* and *Laminaria*, especially in poorly nourished cultures, form gametophytes whose vegetative system is limited to one single cell which forms oogonia. Should this vegetative cell now be omitted in the development, so that the spores of the sporophytes fuse directly, and, moreover, should heterospory be added, then indeed the *Fucus* type is reached. The *Fucus* plant is, then, homologous with the *Laminaria* sporophyte, and also with the *Dictyota* sporophyte, but not with its gametophyte, as HARTMANN (21) and FRITSCH (17) incorrectly assume. *Fucus* "eggs" are therefore from one point of view to be regarded as spores (macrospores), while the spermatozoids are to be thought of as microspores, which, however, become gametes directly without the intermediation of a vegetative gametophyte stage. It should be noted in this connection that the Fucaceae are almost the only plants in the vegetable kingdom in which non-motile "eggs" are thrown out of their oogonia to be fertilized outside the mother plant. Everywhere else

non-motile eggs remain in their oogonia (or archegonia). But this behavior of these eggs is capable of easy explanation when we grant their homology with the spores of other Phaeophyceae. We find now that *Dictyota*, *Laminaria*, and *Fucus* form a continuous series of types from the viewpoint of the alternation of generations: *Dictyota* is an alga in which the generations are of equal size and evenly balanced; *Laminaria* one where the sporophyte completely dominates the insignificant gametophyte; and *Fucus* one in which the gametophyte generation has disappeared and the spores are the only haploid stage in the whole life cycle, and they have likewise become gametes directly. As regards the degree of reduction of the gametophyte, *Fucus* is at exactly the same point as the most advanced phanerogams. *Plumbagella*, examined by DAHLGREN (13), forms the most extreme type of development of the embryo sac of angiosperms, and in it the macrospore becomes directly, so to speak, the egg cell of the embryo sac.

The homology of *Fucus* has long been obscure and misinterpreted, chiefly I think because it is a sexual plant. Since the alternation of generations is often characterized as chiefly an alternation between a sexual and a sexless generation, *Fucus* was therefore viewed as the sexual individual in an alternation of generations. It is, however, not permissible to characterize this alternation solely with regard to sexuality, since to a certain degree sporophytes can also be sexual plants. This appears clearly from the heterospory, which is essentially a sex differentiation. I refer in this connection to the explanatory discussions of CHAMBERLAIN (8) and CORRENS (11). Hence the view held by certain authors, for instance COULTER (12), that it is wrong, from the strict standpoint of homology, to speak of "male" and "female" cones in the conifers, since sporophylls are organs in a supposed sexless sporophyte, is absurd. It is plainly this point of view which leads FRITSCH to call the conception of *Fucus* as a sporophyte "the height of absurdity." *Fucus* is a sexual plant of course, a "Gamont," to use HARTMANN'S (20) word, just as the vegetative phase of phanerogams is. This, however, does not prevent it from being, exactly as in the phanerogams, homologous with the spore producing phase of a plant with a morphologically fully developed alternation of generations.

From this it is evident how misleading it is to characterize the alternation of generations as an alternation between sexual and sexless generations. On the contrary, it is a characteristic feature of this evolutionary process that in the course of the increasing disparity of the generations, in the direction of the dominance of the sporophyte, sexuality passes over more and more to the originally "sexless" generation. CORRENS (11) pointed out that it is more correct to speak of fern sporophytes as of "mixed sexuality" than as sexless.

Comparative studies have now shown that there occurs within the Phaeophyceae the same evolutionary trend with regard to the relation between gametophyte and sporophyte that we know in the series from mosses to ferns and seed plants; but in the case of these algae, this trend is not in any sense due to radically changed external conditions. There is, therefore, so far as I can see, no possibility of explaining the reduction of the gametophyte as being entirely due to the migration of plants to land, as is assumed by the migration theory.

What is the biological explanation of this rise and final predominance of the sporophyte over the gametophyte? I take it that this question cannot be answered except by considering the alternation of generations in connection with the reduction division. GOEBEL (18) and OLTMANNS (33) have asserted that it is a matter of indifference to plants when, where, and how reduction division occurs, but such a view seems to reveal an imperfect comprehension of the real nature of the reduction division.

After an account of the various places in which reduction division occurs in the evolution of the Thallophytes, GOEBEL (18) states:

> It is thus no uniform picture which is afforded by the examples quoted. We see, figuratively speaking, that plants are concerned to bring about the event somewhere and in some manner, but they do not seem to care much at what point in evolution this occurs.

In particular, it has been from many quarters sharply emphasized that the cytological cardinal points, nuclear fusion and reduction division, and in general what has of late been called the alternation of nuclear phases, have no connection with the alternation of generations, characterized by HOFMEISTER on a morphological basis.

I do not share this opinion. Those investigators who consider that the position of reduction division in the life cycle of the plant is a matter of secondary importance, have laid too much stress on the rôle of the reduction division in "reconstructing" the double number of chromosomes; but the main significance of the reduction division surely lies rather in its rendering possible new combinations of chromosomes in the daughter nuclei.

In a certain measure reduction is to be regarded as the final act and the goal of fertilization. Very similar diploid nuclei at their respective reduction divisions can give rise to daughter nuclei differing very considerably, according as the chromosomes chance to combine. When the chromosome number of a plant is known, it can easily be calculated how many combinations are possible, and since at every reduction division, owing to the nature of the heterotypic division, two possibilities at most can be realized, it is easy to calculate the theoretically lowest number of reduction divisions required in order that all conceivable combinations may become possible. If the haploid chromosome number of a plant is a and its diploid consequently $2a$, then the number 2^a gives the number of possible different combinations of haploid nuclei, and the number 2^{a-1} is the theoretically lowest number of reduction divisions required in order that all possible combinations shall be able to be realized. These numbers are the same as those we know from the Mendelian hybrid laws for the different combination possibilities of pairs of characters. A plant with ten chromosomes in the haploid nucleus, and consequently twenty in the diploid, can by reduction divisions so combine them that 1024 $(=2^{10})$ different haploid nuclei can be formed, and for this at least 512 $(=2^{10-1})$ different reduction divisions are required.

Since, therefore, different diploid nuclei with exactly the same number of chromosomes are able to carry out reduction division with different results as regards chromosome combinations, it is clear that when a reduction division follows at once upon a fertilization (that is, one fertilization is only compensated for by one reduction division), never more than two chromosome combination possibilities can be realized; but when a more or less highly developed diploid sporophyte is formed, which produces numerous spore

mother cells, each of which undergoes reduction division (that is, when a fertilization is compensated for by many reduction divisions), numerous combination possibilities are sure to be realized. This is no unimportant matter when we are concerned with explaining the origin of the variety and the wealth of form found in the plant world. From this, moreover, it follows that it is not a matter of indifference when, where, and how the reduction division occurs in the life cycle of a plant. There are, broadly speaking, two distinct types of this in the vegetable kingdom. The fertilized diploid nucleus may either immediately undergo reduction division (thus displaying one fertilization, that is, one reduction division as in Conjugatae *Coleochaete*, haplobiontic red algae, etc.), or else the reduction division is postponed and a diploid sporophyte is formed, in which case several or many reduction divisions occur as a result of each fertilization, as in the diplobiontic red algae, mosses, ferns, phanerogams, etc. Not a single type in which reduction division is delayed and a diploid sporophyte or a diploid phase is formed (which compensates for fertilization by one single reduction division) is known in the whole of the vegetable or animal kingdoms, and in all likelihood such a type does not exist, although of course it is theoretically conceivable.

Proceeding from these considerations, the following working hypothesis may be postulated for the biological explanation of the origin of a sporophyte: the development of a diploid sporophyte, due to the delay of the reduction division, secures to the plant the possibility of bringing about numerous reduction divisions and thereby numerous character combinations. The rise of more abundant forms, and indirectly a larger number of fit types, is hereby rendered possible as the result of a fertilization. When, therefore, as in the *Coleochaete*, *Scinaia*, etc., reduction division follows immediately on fertilization, and only one haploid nucleus becomes further developed, all their spores are alike with regard to the assortment of chromosomes and to the inheritance combinations, although numerous propagating bodies, carpospores, are formed. On the other hand, in *Polysiphonia*, where reduction division is delayed and a diploid plant arises, various chromosome combinations can be realized at the different divisions of the many tetraspore mother cells.

The objection may now be raised that this delay of the reduction division is only of significance in hybrids, as a mere new combination of chromosomes would be meaningless unless something new were added. To this it may be replied that "hybridization" is only a special case, the crossing of species, of a universal blending of paternal and maternal properties occurring in all sexual organisms.

All sexually originated organisms are to a certain degree hybrids, if only the gametes are unlike, and in the majority of cases, except in complete homozygosis, they are unlike. Only when one and the same haploid plant forms male and female gametes which fuse with each other are they not unlike. In these circumstances most fertilizations are by gametes from different individuals, hence are to a certain degree hybridizations, and then certainly the delay of the reduction division is significant. One possible objection may be offered to this view, namely, that when in a haploid plant a great number of haploid gametes are formed in the gametangium, and these fuse with one another, this also results in many reduction divisions, and the plant has just as great a possibility of a diverse recombination of its chromosomes as if it formed a diploid generation with numerous spores arising by reduction division. To this the following reply can be given: a plant which is haploid can form in its gametangia only gametes of the same kind. The same diploid plant, however, thanks to the many reduction divisions, can form gametes of different kinds. In the former case, supposing that four different types of gametes (=factors) are present, there must also be four different types of haploid plants present if all the different combination possibilities are to be realized. Now four gamete types can be combined in sixteen different ways and nine genotypically different zygotes formed (cf. the general scheme of factors). Of these, four are homozygotes. In such individuals it is indifferent whether reduction division occurs immediately or is delayed, since they only form similar gametes. Eight zygotes are heterozygotic in one factor. Here at reduction division two different gamete types are formed, and on the supposition that at reduction division all the daughter cells of the tetrad are preserved, and not (as so often happens in nature) that three are destroyed, it is perhaps indifferent whether reduction division occurs at once or is postponed; but how

often does it happen that not all the daughter cells of the tretad survive, most of them degenerating. In such majority of cases a delay of reduction division is manifestly of advantage. Finally, four zygotes are heterozygotic in both factors. If reduction division occurs immediately, two types of gametes at most can be formed. On the other hand, if a diploid sporophyte is formed, followed by several reduction divisions, there is nothing to prevent the formation of all four gamete types as the result of fertilization.

Thus it follows that if reduction division occurs immediately, at least four fertilizations would be required instead of one in order to attain the same result, that is, to produce the four different possible types of gametes. Now, instead of four fertilizations, a single one can attain the same result. In the former case, not only four times as many gametes are necessary (eight instead of two), but also four times as many fusions. If the plant, by the delay of the reduction division, assumes a diploid structure, the originally haploid generation is reduced to the spores, and, finally, even these spores take the character of gametes; exactly as happens in the case of *Fucus*, this must be a far more advantageous organization than the haploid one. In this case (*Fucus*, phanerogams) sexuality has also migrated to the diploid sporophytes, and only the gametes are left as the last morphological remnant of the haploid generation. Thus it is more advantageous to the plant to become diploid than to remain haploid, since the diploid plant is not larger, demands no more room, assimilates as well, etc., as the haploid, and yet can, owing to its diploid structure, produce not only one but several kinds of gametes. What this means, when the ground is occupied by all sorts of plants among whom keen struggle for existence soon arises, may easily be understood. Assuming that for one species unlimited space was available, and further that possibilities of fusion could easily be realized, it would then perhaps be immaterial whether the plants developed as haploids or diploids. It is a fact, however, that everywhere in nature those organisms which can produce the greatest effect with the least expenditure of material gain the victory in the struggle. The diploid organism has done this, and that appears to me to be the reason why everywhere in the highest ranks of the vegetable kingdom the diploid generation predominates.

If this hypothesis be correct, then it must also be true that the first type (one fertilization—one reduction division) is a more primitive and more simple form of plant organization, and that the later type (one fertilization—many reduction divisions) comprises forms which, as is shown by other features, have reached a higher stage of evolution.

In the first type we may include Flagellates, plankton diatoms (*Diatomeae centricae*), Conjugatae, Chlorophyceae, the haplobiontic Florideae, and Phycomycetes. To the second type belong pennate diatoms (*D. pennatae*), diplobiontic Florideae, Phaeophyceae, Myxomycetes, Ascomycetes, and Basidiomycetes, as well as Bryophytes, Pteridophytes, Gymnosperms, and Angiosperms.

A comparison between the red algae where reduction division is immediate, which the writer (**45**) has called haplobiontic forms, and the diplobiontic ones where several reduction divisions occur to each fertilization, shows unmistakably that the greater abundance of forms and species and the higher differentiation of the plant body are present in the latter. It is obvious, moreover, that the now-living Chlorophyceae and Conjugatae, which are almost all haploid, have not succeeded in raising themselves to equality with the more vigorous plant forms. On the other hand, we see in the Phaeophyceae a group that is incontestably the most highly organized and differentiated of all algae. I may mention the Sargassums, which, with their shoot differentiation and their leaf formation, etc., are little behind the phanerogams in shoot organization. The fact that the Phaeophyceae, as it is now proved, have, like the phanerogams, gone farthest among all the algae toward restricting most of their development to the diploid generation, cannot, it seems to me, be altogether an accident.

Fungi point the same way too. The haploid Phycomycetes display a far smaller variety of forms than the Eumycetes (Ascomycetes and Basidiomycetes). Properly speaking these can hardly be termed diploid, but the presence of the curious pairs of nuclei consorting in their cells generation after generation leads to precisely the same outcome, since many reduction divisions finally result from every nuclear fusion.

A comparison shows that wherever several reduction divisions

follow each fertilization, there are to be found more highly organized forms, and above all a noticeably greater variety of forms; that, briefly, this type must be regarded as being decidedly more highly evolved than the other, which is more primitive. The facts of nature also confirm the hypothesis that the origin of the alternation of generations, and therewith the origin of the diploid sporophytes, can be explained biologically as an organization advantageous to the plant for the attainment of numerous reduction divisions.

But how are we to explain the progressive reduction of the gametophyte? I believe it is owing to its having gradually become superfluous, since its function is only the production of many reproductive bodies, gametes, all genetically of equal value, with exactly the same stock of chromosomes; this, however, becomes superfluous when the formation of propagules is combined with the reduction division, and the haploid propagules formed thereby become gametes directly, as has been the actual course of evolution within the Phaeophyceae. The same thing appears from a study of the evolutionary cycle Pteridophytes-Gymnosperms-Angiosperms (final stage *Plumbagella*). The omission of the gametophyte stage denotes a high degree of simplification and abbreviation of the whole life cycle of the plant, without the number of sexual fusions being lessened thereby; instead, the number of reduction divisions has been increased, and these have been combined with the initiation of the propagation bodies.

Thus, from the point of view of the alternation of generations, it may be conceived that plants equipped with sexual reproduction have developed, broadly speaking, from haploid single organisms, haplobionts (Chlorophyceae of the *Spirogyra* type), with only a cytological alternation of generations or a nuclear phase alternation, to haploid and diploid double organisms, diplobionts (*Dictyota*-type, Pteridophytes), with both nuclear phase alternation and morphological alternation of generations, and thence, finally, on to the now diploid haplobionts (*Fucus*, phanerogams), likewise with almost entirely nuclear phase alternation but without a manifest morphological alternation of generations, this having finally disappeared.

On the whole, the history of development shows that plants, after having been originally haploid, have passed on to become dip-

loid. The purely morphological alternation of generations is in appearance a kind of transition stage, lacking at the outset, and at the end once more disappearing. This hypothesis has the advantage of being a general explanation of the rise of the alternation of generations in all groups, independently of their external conditions of life.

This hypothesis is offered as an explanation of the alternation of generations in the vegetable kingdom. I am very conscious, of course, that, like all other hypotheses, it does not answer all the questions which may be raised; but the value of a hypothesis depends, so to speak, upon how many different questions it can bring under a single general point of view. I believe I have shown that if the alternation of generations is recognized as a characteristic of practically all plant groups which have fertilization, and if this view is compared with the new facts which modern genetic research has discovered, we shall get nearer the truth than by regarding the alternation of generations as one of the changes of organization which occurred with the migration of the plant world to land.

Every hypothesis has a limited value, and does not contain the whole truth. On the other hand, it must not be forgotten that every hypothesis which once could claim to be reasonable always preserves some truth in itself, even though in due time it has to yield to a new and better explanation.

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[*Accepted for publication November 3, 1926*]

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NATURE OF THE MULTIPLE SEEDED XANTHIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 369

CHARLES A. SHULL

(WITH NINE FIGURES)

Occurrence

Multiple seeded specimens of *Xanthium* occur rarely in nature, but it has been the writer's good fortune to obtain such material from three different localities during the last fifteen years. The occurrence of these three single specimens is so far apart in time and space as to preclude any genetic connection between them. The first burs of this character ever reported, so far as the writer is aware, were those obtained by Mr. F. F. CREVECOEUR near Onaga, Kansas, in the summer of 1909. The name used by CREVECOEUR for this material was *X. canadense* var. *globuliforme*. He adopted this designation because he believed it was derived from *X. canadense*, on the ground that the second generation produced some plants with simple burs of the *canadense* type. Since this date the genus *Xanthium* has been monographed by MILLSPAUGH and SHERFF (7), who go back to the name *chinense* for this species, and by WIDDER (11), who makes *X. canadense* a synonym of *X. pungens*. If this interesting form is to bear only a varietal name, it would be known as *X. chinense* var. *globuliforme*,[1] or *X. pungens* var. *globuliforme*, according to which synonym we choose for *X. canadense*.

This material ran through three generations, counting as the first generation the original parent, which was found growing in a corn field. There was a strong tendency toward sterility, but the second generation produced some seeds in Mr. CREVECOEUR'S flower garden. The third generation, grown by Miss MEEKER at Ottawa, Kansas, in 1911, was entirely sterile. A few burs of the earlier generations came into my hands in 1914, but the seeds were no longer viable, owing to the death of the hypocotyls. Many of the cells of

[1] The name *X. canadense globuliforme* Crevecoeur has been used for this form by MILLSPAUGH and SHERFF, North American Flora 33: 39. 1922.

the cotyledons were still alive, but no germinations were obtained. A photograph and drawings of these burs were published (9), with some suggestions as to the possible origin and significance of the many seeded burs.

It was not until thirteen years after the first appearance of the type at Onaga that a similar plant was found in nature. In the autumn of 1922, Mr. A. A. HANSEN found a multiple seeded specimen near Richland, in the southeastern part of Rush County, Indiana. This is at least 500 miles from Onaga. The burs were sent to the Field Museum, and reached me through the kindness of Dr. E. E. SHERFF, to whom they had been referred for identification. The seeds were tested and found to be viable. Sixteen seeds were found, and from these sixteen plants were grown during the summer of 1923 in the garden of the Hull Botanical Laboratory. A brief note was published (10) at the time, but no detailed account of the material has been given. The plants were very late in blooming, although the season was of normal length. This late blooming was accompanied by what seemed at the time abnormally vigorous vegetative activity. The stems were of enormous size for cocklebures, fully double the diameter of ordinary individuals, and with lateral branches many feet in length. The only photographs taken of this material were of the full grown plant, which is shown in fig. 1, and the fasciated stems, fig. 7. The general impression given to the observer was that they were a *gigas* type of plant, so far as structure and vigor indicated.

The male inflorescences were only in bud in the last days of August and early September, and the female flowers were quite small. Many of the burs were still immature when severe frosts cut the plants off. Attempts were made to remove some of them to the greenhouse to complete the development, but these were not successful. The plants lost their leaves and died. The crop of burs was therefore small, and most of them prematurely ripened. Examination of hundreds of burs, the largest and best that could be selected, showed almost complete sterility. The involucres were much more deeply cleft than those of the original parent plant, and fewer floral cavities developed.

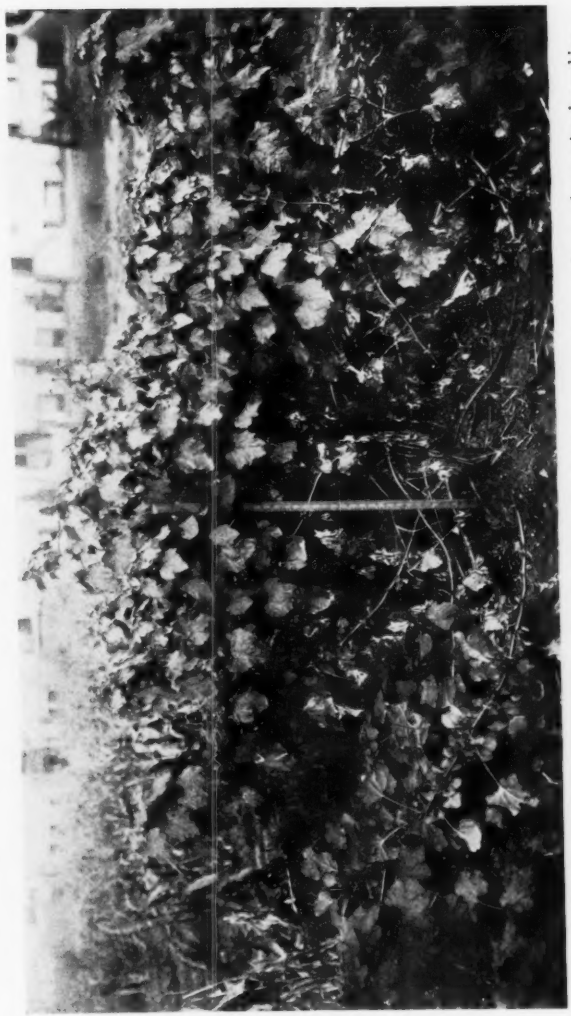

FIG. 1.—Mature plant, from second generation of the Indiana material; note enormous vigor and excessive branching.

Some months later the material was examined by Dr. R. O. EARL, who found after prolonged search a few small, shriveled seeds. From these the third generation was grown late in the summer of 1924. Although the plants were grown in the greenhouse, they began to bloom at about six weeks from the seed, at the same season of the year as the previous generation in the field. The plants ripened off

FIG. 2.—Original stem of Kansas City specimen, 1925

soon after flowering, and none of the burs contained seeds. Attempts to prolong the vegetative life by rooting cuttings were unsuccessful, although the cocklebur can be propagated in this way during the early part of the growing season. The cuttings of this late summer generation, however, continued to ripen their tissues, and died without entering into a vegetative growth period. A higher nitrogen supply might have made a difference in the growth behavior, es-

pecially if it had been accompanied by a longer day, which is known to favor vegetative activity. Thus the material of this second discovery perished without establishing a strain of many seeded burs.

Fortunately a third specimen of the same type was secured in 1925. This specimen was found in the latter part of September by Mr. O. C. DURHAM, at the corner of 70th and Indiana, Kansas City, Missouri. The plant was still green, but the burs were already brown. The specimen was preserved with the burs on the stem. The general appearance of this parent stalk with the original burs is shown in fig. 2. I am greatly indebted to Mr. DURHAM for the specimen, and for having the photograph made. As the second generation has produced an abundance of seeds for a third generation, an account of the habits as observed during the last few years will be given.

Observations on growth

On opening some of the burs from the specimens of fig. 2, well developed seeds were found. There are usually about six good seeds in each bur, but as many as eleven have been found. The seeds are usually found in the outer row of ovarial cavities, but on several occasions good seeds have been obtained from interior cavities. In one of these burs with ovarial cavities for about sixteen seeds, the six good seeds shown about natural size in fig. 3 were found. All were from the outer circle of florets. The seeds are smaller and narrower than those of the common species, and have a different form. They are triangular in cross-section, with one side of the triangle tangent to the outer wall of the involucre. This form is the result of compression of the developing seeds. The seeds are not distinguishable by shape and size as uppers and lowers, nor by position in the burs. Germination of 100 per cent of the seeds on several occasions indicates little tendency to delayed germination, but Dr. E. L. REED reported one seed that did not germinate until its coat had been broken.

FIG. 3.—Six seeds from single bur of plant in fig. 2.

The six seedlings developed from the six seeds in fig. 3 are shown at an early stage in fig. 4. The photograph was taken May 10, 1926.

FIG. 4.—Seedlings of multiple seeded *Xanthium*

FIG. 5.—Specimen of multiple seeded *Xanthium* grown at Stephenville, Texas, July 14, 1926.

In order to insure a longer season of development, some of the seeds were sent to the John Tarleton Agricultural College, Stephenville, Texas. Through the kindness of Dr. REED and his gardener two plants were raised to maturity. Several other plants were accidental-

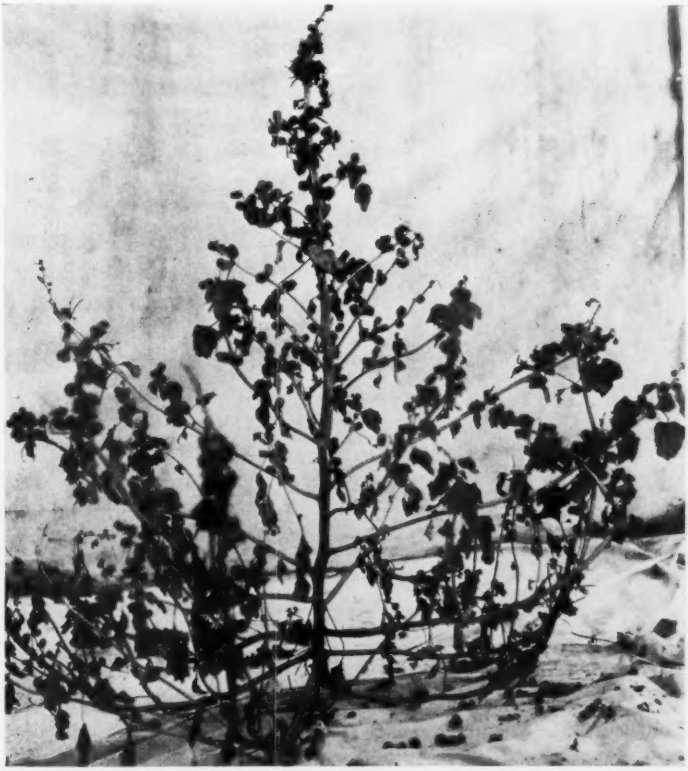

FIG. 6.—Mature plant with ripe burs, Stephenville, Texas

ly destroyed through weeding operations. The plants grew vigorously, as is shown by the photograph, fig. 5, which was taken on July 14, 1926. The precaution of sending this material to Texas was found to be unnecessary, for it proved to have a much earlier blooming time than the offspring of the Indiana material of 1923. The

twenty-five specimens grown in the greenhouse and garden at Chicago also bloomed early enough to ripen thoroughly before frosts.

A full-grown mature plant with ripe burs, at the close of the season, is shown in fig. 6. The general habits of growth are beautifully shown in the photograph. The plants are large and vigorous, with very long lower branches, and numerous branches per plant when grown in the open. In the greenhouse and garden at Chicago all the plants were grown in pots, so that removal to the hothouses would be possible in case of late flowering. The soil mass was not sufficient for maximum growth, and these specimens were by no means so large as the one shown in the figure, but all produced good plants.

FIG. 7.—Fasciated stems of multiple seeded *Xanthium*, from Indiana material in second generation.

Fasciation

A remarkable observation was made in connection with the material sent from Indiana by Mr. HANSEN. Of the sixteen plants grown to maturity, about 75 per cent of them showed a pronounced flattening of the stem, and those that did not show fasciation were excessively branched. This may properly be considered the morphological equivalent of fasciation. Parts of several stems that showed strong fasciation are shown in fig. 7. Examination of the stems failed to disclose injuries which might have caused this. The condition of the parent plant is not known, but in the third generation the fasciation was apparently inherited by all of the specimens. These were grown in the greenhouse where insect injuries were never observed.

The 1926 offspring of the specimen found in Kansas City in 1925 have not shown as pronounced fasciation as the Indiana material. However, if one examines the tip of the stem in fig. 2, it is found to be distinctly flattened, although the lower parts are more nearly terete. In the absence of the writer from Chicago in the late summer and autumn of 1926, the twenty-five specimens grown in the greenhouse and garden were examined by Professor C. J. CHAMBERLAIN, who expressed the opinion that practically all of the specimens showed some signs of being fasciated. In some cases the plants were broadly flattened. It seems, therefore, that fasciation is prominently associated with the multiple seeded condition. The possible significance of this will be considered in the discussion of the causes of the many seeded type.

Photoperiodism

In common with various species of *Xanthium*, the multiple seeded form exhibits a certain amount of responsiveness to photoperiods. Some years ago a number of species were grown at the University of Kansas, and it was observed that each species tended to have its own blooming time. This behavior was spoken of as physiological isolation (**8**). The interesting studies of GARNER and ALLARD on photoperiodism were not available at that time. It is clear now that the *Xanthium* species have the same reason for different blooming periods as, for instance, soy beans, which were found to be strongly photoperiodic.

When the multiple seeded strain from Indiana began blossoming very late in the summer (September), it was thought to be a photoperiodic peculiarity. The material was believed to have the shortest photoperiod of any of the species of *Xanthium* which had been grown. The generation raised by EARL, in the summer of 1924, blossomed at the same time in September as those which were planted in May, 1923, which indicates an inherited tendency. However, there is evidence that material of other origin may have a very different blooming response. The Kansas City material sent to Texas had distinctly visible male inflorescences on July 8, 1926, nearly two months earlier than the Indiana specimens; those raised in the garden and greenhouses at Chicago also showed a much earlier

blooming period. Each strain of the multiple seeded form appears from this to have its own individual blooming behavior.

That the control of blooming in *Xanthium* is not exercised by photoperiod alone was first observed a number of years ago. Seedlings started in the hothouse (25°–27° C.) developed an apical cluster of male flowers in about two weeks from the seed; but when grown in a colder house (10° C. at night) the plants came to blooming in about four months. As the photoperiod was the same for both at the start, it was evident that temperature greatly modified the blooming response. GILBERT (3) has since made a study of this behavior, comparing the metabolic conditions of short-day high-temperature and long-day low-temperature plants. He found a rising carbohydrate-nitrogen ratio as the plants approached the blooming period; but the value of the ratio was lower in the plants that bloomed very quickly from high temperature than in those that blossomed only after a long growth period at lower temperatures.

If there is such a thing as a vegetative-reproductive nutritive equilibrium in plants, it seems to be reversible in *Xanthium;* for if a plant which has been induced to blossom in short-day high-temperature conditions is removed to long-day low-temperature conditions before the maturity of tissues has progressed too far, the plant will become once more vegetatively active by developing new lateral branches. The blossoms already formed may produce seed, but the new axillary branches grow vegetatively a couple of months and come to blossoming at the usual time. In this way we have succeeded in producing two periods of blooming, months apart, in a strictly annual plant.

The multiple seeded forms will probably show the same behavior in every detail. Seeds set to germinate March 11, and planted in pots in the warm greenhouse (25° C.) on March 14, 1927, had visible terminal male flower clusters in nine days from the time of planting. When kept in a box to control the day length to seven hours, the development of the precocious inflorescence was a little more pronounced than when the natural day length was given.

Five of the multiple seeded plants are shown in fig. 8. The smallest seedling, no. 1, was seventeen days old at the time it was photographed. It had been kept in the cold greenhouse, regulated to

10° C. at night. The two plants next to the right are the same age, but grown in the hothouse. No. 2 was grown with a 7-hour day, while no. 3 had the full length day of early April. The two plants at

FIG. 8.—Temperature response: 1, in cool house 17 days; 2, warm house 7-hour day; 3, warm house full day; 4, warm house 7-hour day, 36 days old; 5 warm house full day; plants 2 and 3 are in bud, and 4 and 5 ready to shed pollen.

the right were thirty-six days old, no. 5 having had the full length day and no. 4 a 7-hour day. These plants had just one cluster of male flowers, the terminal cluster, and the female flowers occupy all of the leaf axils except those of the cotyledons. In fig. 9 is shown the leaf display of the 7-hour plant no. 4.

FIG. 9.—Vertical view of plant no. 4, fig. 8, showing leaf development and terminal male inflorescence; plant 33 days old.

Plant no. 5 began to shed pollen on April 12, at thirty-two days, about twenty-three days after the buds first became visible. Plant no. 4 shed its first pollen on April 18. The short day apparently retards development at this particular season. It is seen also that the full-day length plants have longer internodes than the short-day plants.

It is remarkable that plant no. 4, almost ready to bloom, still has its cotyledons in active condition. The cotyledons on plants in full-day length shriveled much sooner than those in the 7-hour day.

As the day length increases, the tendency to precocious blooming decreases, and finally ceases altogether. This behavior is identical with that observed by GILBERT in the common species of *Xan-*

thium. During the longer day season, flowering can be induced by shortening the length of the illumination period, in plants kept at high temperatures. Temperature and photoperiod seem to be about equally important in determining the behavior of the organism.

Possible causes of multiple seeded condition

Originally the writer was inclined to believe that the multiple seeded bur was the result of a reversional mutation toward remote ancestry; that the many seeded capitulum represents the type of inflorescence in the progenitors of the cockleburs of this age. From this many seeded bur our two seeded burs have come by a process of reduction, if we accept FARR'S (2) interpretation of the inflorescences of *Xanthium*. Such reversions to ancestral floral conditions have been noted in other species of Compositae, as by COLLINS (1) in the case of hybrid forms of *Crepis*. This interpretation should be kept in mind, for it is a logical explanation of the phenomena.

There has always been a question as to whether hybridization might be a factor in the production of this type of plant. Although CREVECOEUR'S observations of the multiple seeded species of *Xanthium* in 1910 indicated a hybrid condition (9), such splitting into groups of many seeded and 2-seeded forms has never been observed in any of my cultures. The inheritance of the multiple seeded condition has been perfect in every generation thus far grown. Self sterility in the first two strains might be thought to indicate hybrid condition. JEFFREY (4, 5) has championed the idea that self sterility is a mark of hybridization, but it is conceivable that other causes might be responsible for it, as for instance the fasciated condition observed in these forms.

Indeed, one is led to inquire whether it may not be possible that the multiple seeded condition is merely the expression of fasciation, carried out into the burs. Is one of these burs simply an aggregation of a dozen or so burs into one, a fasciated bur? Investigations have not gone far enough along this line to answer this question, but the way seems open now to test certain phases of the matter experimentally. Miss JOHNSON (6) has recently shown that *Helianthus annuus* can be caused to fasciate its stems, leaves, and flowers by treatment with the X-ray. Since some other Compositae have also

shown a tendency to fasciate after exposure to X-rays, Miss JOHNSON has undertaken to determine whether the ordinary species of *Xanthium* with two seeds to the bur can be caused to fasciate in this way, and whether such induced fasciation leads to the production of multiple seeded female inflorescences.

While it is possible, therefore, that we have here a mutational reversion to a far distant ancestral condition of inflorescence, the fact of the association of fasciation with the multiple seeded condition of the bur should lead to caution in the interpretation. How far can we go in linking sterility with fasciation is a question. At present we know little about the cytological details of pollen formation and the development of the female gametophyte in the ordinary species of *Xanthium*. Chromosome behavior during cell division in normal and fasciated meristems has never been studied. Fasciation may not be accompanied by any spindle irregularities, or chromosome aberrations, but it is possible that it is. The morphological and cytological details of the meristems of these different types of growth forms should be known before we attempt to go any further with the interpretation. The chromosome numbers of the normal and multiple seeded forms should be known, to determine whether the vigorous growth of the many seeded type is at all associated with a doubling of the chromosome number, as in the *gigas* Oenotheras. No such doubling is expected in this case, but the facts should all be known. The last specimen obtained from Kansas City has provided a great supply of material suitable for such morphological and cytological studies.

Summary

1. Multiple seeded specimens of *Xanthium* have been found in nature on three occasions, in 1909, 1922, and 1925.

2. The first two groups, after being grown for two generations following the original plants, perished because of infertility.

3. Plants grown in 1923 showed very marked fasciation, which was exhibited by 100 per cent of the two generations. It is believed to be strictly inherited. The 1925 material showed less pronounced fasciation in the generation grown in 1926, but nearly all specimens showed evidence of this modification.

4. It is suggested that the fasciation may have something to do with the partial sterility of this variety of *Xanthium*.

5. The multiple seeded condition may have arisen as a reversion toward remote ancestral floral conditions, or it may be associated with the fasciation. In the former case, the bur would represent a many-flowered capitulum from which our present two-flowered capitulum has been derived by reduction. In the latter case, the bur would represent a dozen or more burs consolidated into one through fasciation.

6. This form of *Xanthium* exhibits the same peculiar photoperiodic and temperature responses as the common species of the Chicago region. The vegetative-reproductive equilibrium seems to be reversible, as an induced blooming period may be followed by vegetative activity and a later blooming period of the vegetatively formed branches.

7. The multiple seeded variety of *Xanthium* presents some unique and interesting problems of anatomy, cytology, and floral development.

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[*Accepted for publication April 20, 1927*]

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ANATOMY AND DEVELOPMENT OF TOMATO FLOWER[1]

DELMER C. COOPER

(WITH PLATES XI, XII AND SEVEN FIGURES)

The literature of recent years concerning the tomato, *Lycopersicum esculentum* Mill., contains the reports of many investigations in the fields of pathology and physiology, and comparatively little about the morphology of the plant. Study of the morphology and anatomy has been secondary to that of nutrition and disease.

For the investigation of the anatomy and morphology of the flower here reported, two well known varieties were used, Bonny Best and Greater Baltimore, since these could be obtained in abundance in the greenhouses of the Purdue Agricultural Experiment Station.

Gross morphology

The flower cluster of the tomato is a short, forked racemose cyme of seven to twelve flowers (fig. 1). It is unusual for more than two flowers to open at one time in each inflorescence, so that a peduncle is often found bearing small fruits, open flowers, and developing buds at the same time (fig. 2). The tomato flower is perfect, hypogynous, regular, pendant, and typically six-merous (fig. 3). The calyx tube is very short, and bears six leafy lobes which are linear to lanceolate (fig. 4). The calyx is persistent and increases in size with the development of the fruit. The rotate corolla consists of a short supporting tube and six broad lobes. It is a pale greenish yellow when the flower is partly opened, and the thin lobes are much wrinkled. In the fully expanded flower the lobes are much reflexed and a brilliant yellow.

The six or more orange-yellow stamens (fig. 21) are attached adnately to the throat of the corolla tube, and are connivent or syn-

[1] Contribution from the Biological Laboratories, Purdue University, Lafayette, Indiana. A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science.

egesious, that is, they are laterally coalesced to form a hollow cone around the pistil, which occupies the central portion of the flower.

The inconspicuous pistil is pale yellowish green, and consists of the enlarged basal portion or ovary, the elongated style, and the rather flattened stigma which extends slightly beyond the apex of the androecium (fig. 18). The pistil is composed of six fused carpels

FIG. 1.—Flower cluster showing pendant nature of flowers; natural size

and the ovary is typically six-celled. The corolla and androecium fall off soon after pollination, and the style follows not long afterward.

It is of interest to note that BAILEY (1), GRAY (4), and PAYER (7) describe as five-merous the whole order Solanaceae. THOMPSON (9) reports the same condition in his description of the tomato itself, and BRITTON and BROWN (2) consider the flower as being five-merous, rarely six-merous. On the other hand, many workers, not especially concerned with the morphology of the flower, have recorded the six-merous condition.

Examination of more than 1000 flowers showed that the varieties studied are basically six-merous. The first 200 flowers revealed two cases of numerical variation due to a multiplication of parts. Only one of the blossoms out of the total number examined was found to be five-merous throughout. During the summer of 1926 the flowers on tomato plants in many gardens were examined, and were found to

FIG. 2.—Peduncle bearing very young fruit, mature flowers, and unopened flower bud; abscission region can be seen on pedicels of several flowers; natural size.

be typically six-merous. It was discovered, however, that the flowers of the pear-shaped tomato, *L. pyriforme*, are five-merous.

Development of floral organs

For the microscopic study, developing flower buds in various stages of growth were gathered from healthy plants, fixed in a solution made by adding 6.5 cc. of formaldehyde and 2.5 cc. of glacial acetic acid to 100 cc. of 50 per cent alcohol, dehydrated, cleared in chloroform, and imbedded in paraffin. Both cross and longitudinal sections were cut, 12 μ in thickness, mounted serially on slides, and stained in safranin and Delafield's haematoxylin. Some small buds and parts were similarly fixed, stained, dehydrated, cleared in xylol, and mounted *in toto* for a study of the gross anatomy.

In the development of the flower, a small protuberance of meristematic tissue grows out from the pedicel of the preceding flower. The portion of this pedicel posterior to the protuberance becomes part of the peduncle. In the case of the first flower of the cluster,

this meristematic protuberance or axis grows from the axil of the leaf. The first primordium of the floral organs develops on one side of the meristematic protuberance when the latter is of microscopic size. Other primordia develop, one after another, passing around the protuberance in a clockwise manner until a ring of six (fig. 19) is formed on the circumference. By the time that this cycle is completed the first primordium has developed to some extent, and the others in proportion, so that each is somewhat longer than the one subsequently produced. Growth of each of the first six rudiments is more rapid on its dorsal side, so that concave structures are produced which arch over the apex of the axis (fig. 19). Thus the calyx forms a protective covering over the growing tip of the protuberance, the apex of which becomes the receptacle.

FIG. 3.—Tomato flower showing six-merous condition; natural size.

The primordia of the second whorl grow out in the same manner, except that they are slightly higher on the axis and are alternate with the calyx primordia. The second whorl develops into the corolla. Next appears the third or staminal whorl, the primordia of which are opposite those of the first whorl and alternate with those of the second. Similarly, the primordia of the fourth whorl, which develops into the pistil, are alternate with those of the preceding one (figs. 20, 21). The floral organs are thus developed in a low spiral arrangement of four turns around the receptacle, the fourth whorl forming the apex.

The development of the primordia of the outer circle is such that they broaden at their bases until they touch one another laterally, after which the entire ring of meristematic tissue grows upward, forming a low cylindrical calyx cup. The calyx, therefore, is not

formed of sepals which were originally free and later united laterally at their bases, but rather is formed by the outgrowth or protrusion of a ring of meristematic tissue basal to the early whorl of six distinct and separate primordia.

Protected by the developing calyx, the second whorl of primordia, the forerunners of the corolla lobes, develops slowly in comparison with the other whorls of the flower bud. The formation of the corolla tube is similar to that of the calyx tube. Just before maturity of the bud the corolla makes rapid growth, and finally pushes the calyx open. As the corolla tube grows out from the receptacle, the six meristematic regions of the third or staminate whorl of primordia grow up with it in such a way that the stamens are adnate to the interior of the corolla tube, and appear to have developed from it.

FIG. 4.—Young inflorescence showing hairy condition and position of flower cluster at apex of plant; ×10.

Each primordium of the staminate whorl or androecium consists of homogeneous tissue, which, as growth continues, becomes differentiated into distinct parts. The apical portion of each stamen becomes the anther, and consists of four lobes of sterile tissue and sporogenous cells. The basal portion develops into an elongated filament adnate to the corolla tube, except for the rather thick free stalk which terminates in the anther.

The fourth whorl of six primordia develops into a compound pistil consisting of a group of six united carpels. The placenta on the inner or axillary wall of each carpel enlarges, so that it, with the developing ovules, nearly fills the locule (fig. 16). The development of the ovary is uniform from the first.

Anatomy of mature floral organs

PEDICEL.—The pedicel which supports a single flower, as well as the peduncle from which it arises, is composed of a rather thick cortex, a ring of vascular tissue, and a central portion of pith (*fig. 22*). The epidermis of the pedicel is composed of long narrow cells covered with a cuticle, and contains relatively few stomata. These are arranged in such a way that the stomatal openings are parallel to the long axis of the pedicel (fig. 9). Long multicellular non-glandular hairs, and short stalked glandular hairs tipped with four cells are scattered over the surface of the pedicel (figs. 11, 12, 13). The abscission layer (*fig. 5*) of the flower is formed about midway in the pedicel, or about 1 cm. below the receptacle. At this point the pedicel is somewhat constricted, and the individual cells are small and much flattened. These small cells later become more or less corky, and finally separate with the ripening of the fruit, so that the pedicel breaks at this point.

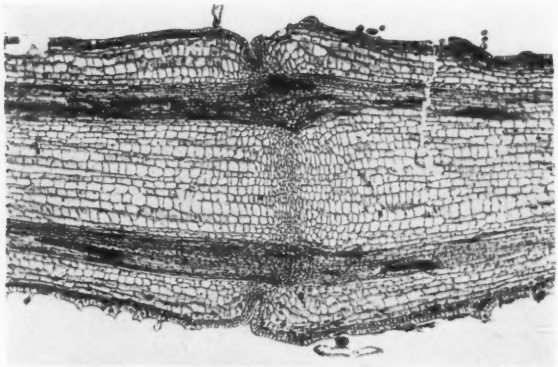

FIG. 5.—Photomicrograph of longitudinal section of pedicel showing abscission layer; ×40.

The receptacle becomes a rather large, very flat disk, within which the vascular cylinder divides into strands which lead to the various organs of the flower (*fig. 14*). These strands or bundles radiate outward, one to each of the floral parts; consequently bundles leading to the corolla lobes are opposite the sinuses of the calyx.

CALYX.—The first six vascular bundles leading from the vascular cylinder in the receptacle radiate outward, one to each lobe of the calyx (fig. 24). Each bundle becomes a finely branched and anastomosing system of veins leading to all parts of the lobe. The cellular structure of the calyx lobes is similar to that of the leaf. Each lobe is about fifteen cell layers in thickness through the midrib. The mesophyll is rather spongy, and its cells contain many chloroplasts. The epidermis is composed of a single layer of small cells covered with a thin cuticle.

Stomata are found in great abundance on the dorsal surface of the calyx, especially toward the base of each lobe and on the calyx tube. These stomata protrude above the surface of the epidermis (figs. 8, 15). Those on the ventral surface of the calyx are small, and are not raised above the general surface. An intercellular space or substomatal cavity is formed under each stoma.

Several types of hairs are found on the surface of the calyx. Those on the dorsal (lower) surface vary from short unicellular hairs to long multicellular hairs (figs. 11, 12). Many of the longer hairs are supported by a group of cells which protrude above the general surface of the calyx. The glandular hairs (fig. 13) are similar to those occurring on the pedicel, except that the stalks of the former are longer. The single celled hairs are simply an outward extension of individual epidermal cells. Papillae and short stalked hairs are found on the ventral surface of the calyx lobes. The calyx, unlike the corolla and androecium, which disappear soon after pollination, is persistent and continues to grow.

COROLLA.—The second zone of six vascular bundles originates in the vascular cylinder of the receptacle just above the first zone, and enters the corolla, a single bundle passing into each lobe (figs. 25, 26). The epidermis of the corolla is composed of a layer of thin walled cells, some of which on the ventral surface, especially at the apex of the lobes, protrude out into papillae (fig. 10). Many glandular hairs with very short stalks are present on the dorsal surface. No stomata were observed on the corolla. The lobes of the corolla are about eight cell layers in thickness, and consist largely of spongy mesophyll. The color is due to yellow chromoplasts within the cells of the mesophyll.

ANDROECIUM.—The third zone of six vascular bundles originates in the vascular cylinder of the receptacle, just above and alternating with those of the second whorl (figs. 27, 28). The stamens are carried upward with the growth of the corolla tube. The short free portions of the filaments thus seem to have developed from the throat of the corolla, rather than from the receptacle; however, the vascular trace of each stamen passes as an unbranched bundle from the receptacle through the adnate filament to the anther. Each anther is divided longitudinally into a right and left lobe, separated by the connective tissue. Each lobe contains two microsporangia which extend approximately the full length of the anther. At maturity the dorsal side of the anther is covered with large papillae. The ventral side has a few hairs, especially at the apex. There are no hairs along the line of dehiscence. With the exception of the pollen grains and the vascular bundle, each stamen consists of parenchyma cells surrounded by an endothecium. Stomata apparently are lacking on all parts of the stamen.

Although the flower (figs. 20, 21) is typically six-merous throughout, individual flowers occur which possess a variable number of stamens. In cross-section it was occasionally found that one or more filaments might contain two vascular bundles. Such filaments were surmounted by two anthers. The two vascular bundles of such filaments, however, are found to result from a branching of one of the six bundles arising from the stele of the receptacle (figs. 28–31).

Four microsporangia, two to each lobe, are formed in the anther, and extend approximately its full length. In cross-section each microsporangium appears somewhat horseshoe-shaped (fig. 6). After the development of spore mother cells from the sporogenous cells, the conjunctive or sterile tissue separating the sporangia of each lobe breaks down, leaving one spore chamber (pollen sac) in each lobe (fig. 7). Later each spore mother cell divides into four cells, each of which is a microspore. The microspores develop thickened cell walls and become pollen grains.

When the corolla begins to open, the stamens are pale yellow, and the spore chamber is closed. After a period of 24–48 hours, depending on atmospheric conditions, the color of the stamens changes to a bright yellow and dehiscence begins. This is accomplished by a longi-

tudinal splitting of the spore chamber wall along its weakest part, which is the place where the conjunctive tissue has recently broken down. This splitting is introrse, so that the pollen grains fall on the stigmatic surface of the pistil.

PISTIL.—The vascular bundle extending into each carpel divides, so that one branch passes outward and upward in the pericarp, and

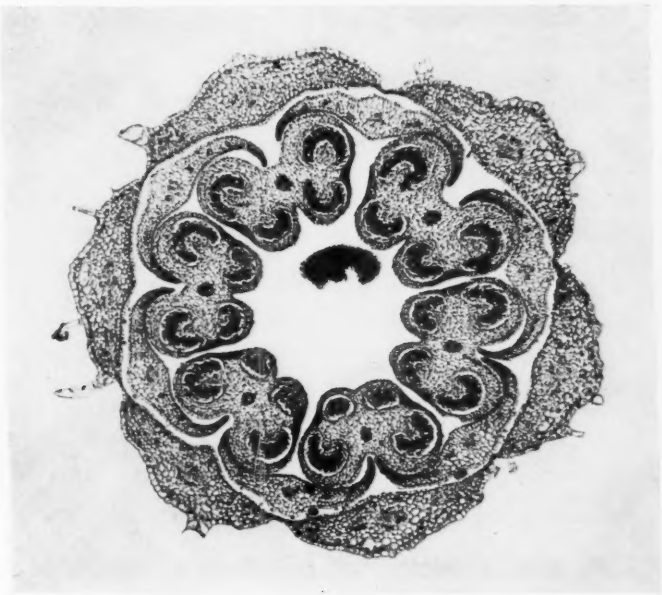

FIG. 6.—Photomicrograph showing six-merous condition of flower bud as seen in cross-section; ×50.

continues up through the style to the stigma (figs. 28, 30), while the other passes through the placenta and divides into many smaller bundles, each one ending in an ovule (fig. 31). The papillae of the stigmatic surface secrete a sticky fluid in preparation for pollination.

Small papillae similar to those on the corolla (fig. 10) are scattered over the epidermis of the ovary, and extend well up on the style. Large papillae and multicellular glandular and non-glandular hairs are found on the basal half of the style and on the ovary.

Rather large stomata are sparsely scattered over the style, but none could be found on the ovary. MAKEMSON (6) reported stomata and lenticels on the ovary, but GROTH (5), ROSENBAUM and SANDO (8),

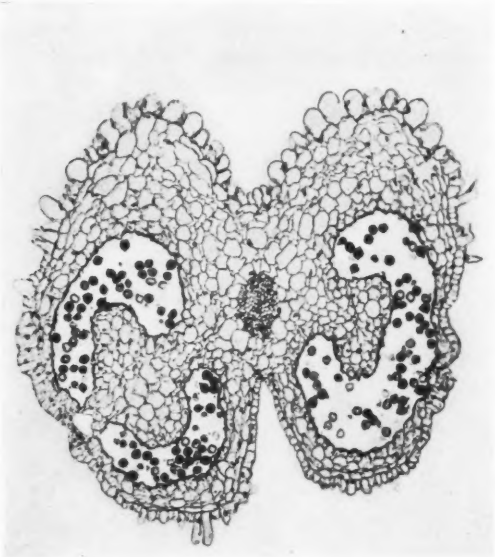

FIG. 7.—Photomicrograph of cross-section of anther showing mature pollen grains and disappearance of conjunctive tissue; ×85.

and GARDNER (3) found no stomata on the ovary, although the last named, like the writer, noted their presence on the style, receptacle, calyx, and pedicel.

Summary

1. The inflorescence of the varieties of tomatoes studied is a racemose cyme bearing 7–12 flowers.

2. The floral organs are developed in a low spiral arrangement of four turns around the receptacle. The six primordia of the calyx develop successively, passing around the receptacle in a clockwise manner. The primordia of the succeeding cycles develop in a like manner, except that they alternate with those of each preceding cycle.

3. Each zone later becomes completely meristematic, so that the floral organs of each whorl are laterally coalesced, so to speak. Finally the staminal and corolla zones become radially and laterally one continuous zone, so that the stamens appear to be adnate to, or outgrowths from, the corolla tube.

4. Each calyx lobe, corolla lobe, stamen, and carpel is supplied with one bundle from the vascular cylinder in the receptacle.

5. The calyx consists of a short tube surmounted by six linear to lanceolate lobes, 12–15 cell layers in thickness. The mesophyll is loosely arranged, and contains numerous chloroplasts.

6. The corolla is a short tube bearing six broadly lanceolate lobes, about eight cell layers in thickness.

7. The six stamens are connivent, and form a cone around the pistil. They may be said to be inserted on the throat of the corolla, but their primordia are at first free from it. Dehiscence is introrsely longitudinal.

8. The pistil is composed of six united carpels.

9. Glandular hairs are found on the calyx and corolla. Multicellular non-glandular hairs occur on the calyx, corolla, and style. All of the organs bear single celled papillary hairs. Stomata were found on the calyx, pedicel, and style, but not on the corolla or ovary.

The writer feels deeply indebted to Professor L. F. HEIMLICH for his generous assistance during the course of this investigation; to Professor E. J. KOHL who made the photomicrographs; and to Dr. M. W. GARDNER, through whose courtesy the material for study was obtained.

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[*Accepted for publication January 10, 1927*]

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EXPLANATION OF PLATES XI, XII

FIG. 8.—Arrangement of stomata on dorsal surface of calyx lobe; ×250.

FIG. 9.—Arrangement of stomata on pedicel; ×250.

FIG. 10.—Papillae on surface of corolla; those on ovary similar; ×250.

FIGS. 11, 12.—Multicellular non-glandular hairs from dorsal surface of calyx; ×250.

FIG. 13.—Multicellular glandular hair from calyx; ×525.

FIG. 14.—Median longitudinal section through flower bud, drawn from fresh material; ×5.

FIG. 15.—Cross-section of calyx lobe, showing elevated stoma and substomatal cavity; ×250.

FIG. 16.—Longitudinal section through ovary, showing locule and forking of vascular bundle (shaded); ×72.

FIG. 17.—Stamen in dorsal, ventral, and side views drawn from fresh material; ×3.

FIG. 18.—Pistil showing six united carpels; ×3.

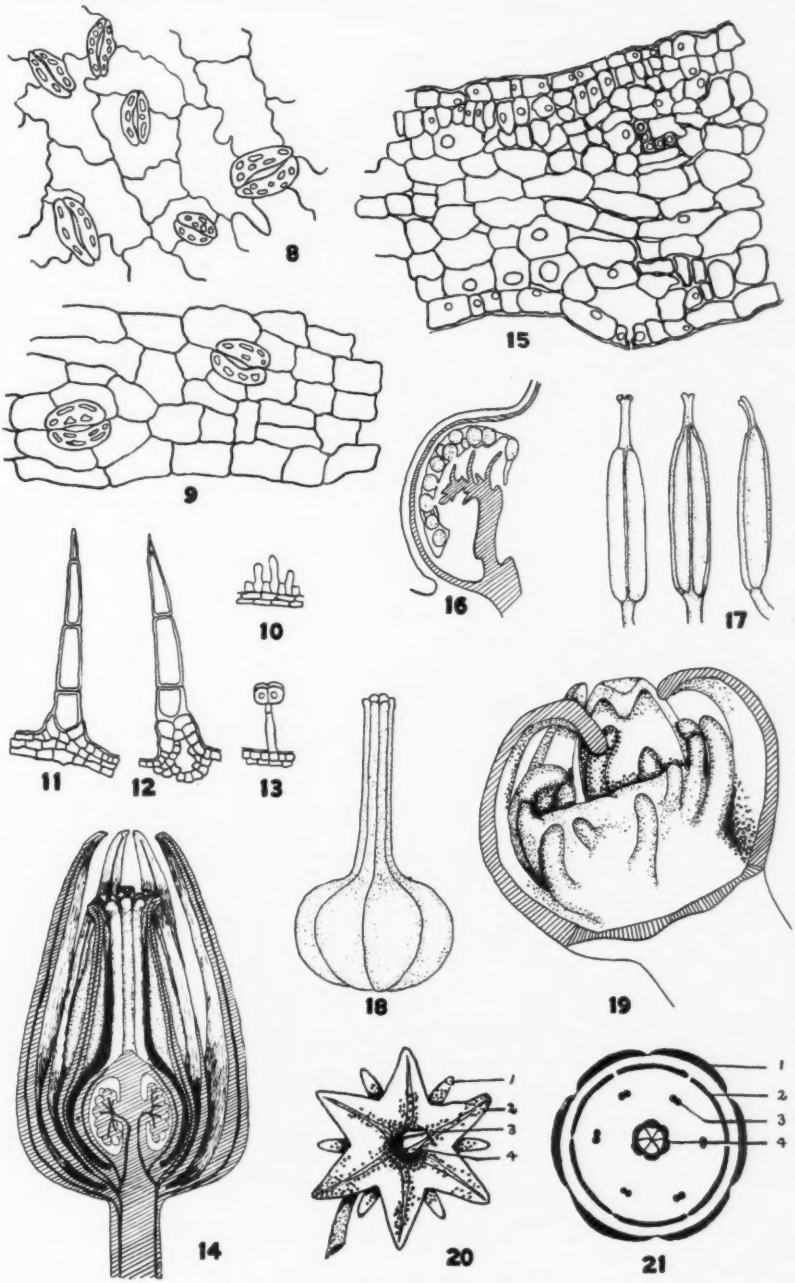
FIG. 19.—Young flower bud with part of calyx removed to show developing primordia; reconstructed from series of drawings of longitudinal sections; ×125.

FIG. 20.—Tomato flower showing six-merous condition; drawn from fresh material.

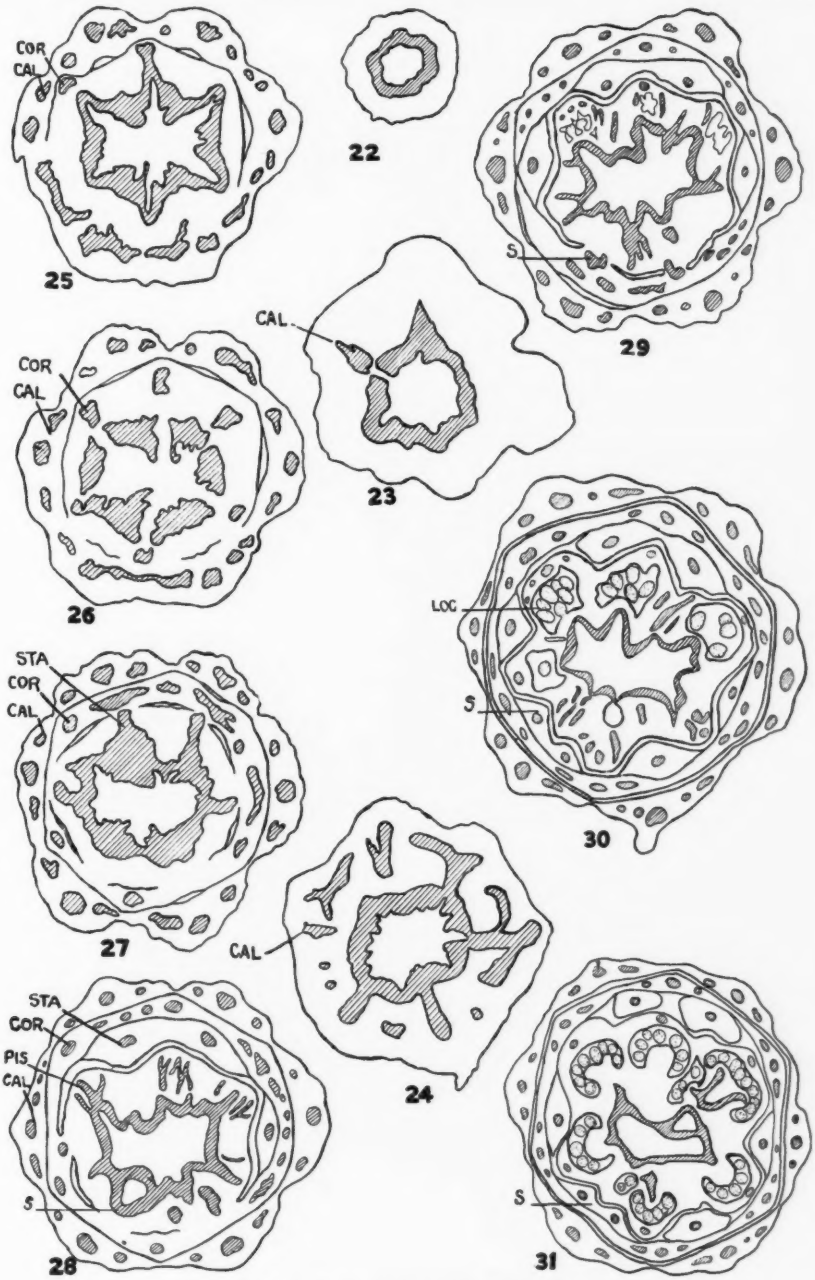
FIG. 21.—Diagrammatic plan of tomato flowers: 1, calyx lobe, 2, corolla lobe, 3, stamen, 4, carpel.

FIG. 22.—Cross-section of pedicel just below receptacle, showing vascular cylinder (shaded); ×12.

FIGS. 23, 24.—Cross-sections through base of receptacle, showing origin of vascular bundles (*cal*) leading to calyx lobes; fig. 3 shows branching of each bundle; ×12.



COOPER on TOMATO FLOWER



COOPER on TOMATO FLOWER

FIGS. 25, 26.—Cross-sections through receptacle just above traces leading to calyx, showing origin of traces (*cor*) leading to corolla; ×12.

FIG. 27.—Vascular traces (*sta*) leading to staminal whorl; ×12.

FIGS. 28, 29.—Vascular bundles (*pis*) leading to style and stigma; ×12.

FIG. 30.—Locules (*loc*) formed in ovary, but vascular cylinder still intact; ×12.

FIG. 31.—Vascular cylinder breaking up into branches leading to ovules; ×12.

FIGS. 28, 29, 30, 31.—Series of sections showing at *S* development of two vascular bundles within one filament; ×12.

SMOOTHNESS AND ROUGHNESS AND SPONTANEOUS AGGLUTINATION OF *BACTERIUM CITRI*, BACT. MEDICAGINIS VAR. PHASEOLICOLA, BACT. PHASEOLI SOJENSE, AND BACT. TUMEFACIENS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 370

G. K. K. LINK AND KATHLEEN L. HULL

(WITH THREE FIGURES)

In the course of routine cultural work, and of observations incidental to agglutination tests of a series of experiments by LINK and LINK (6), and LINK and TALIAFERRO (7), data were obtained which appear significant in connection with the discovery by SHARP (10) of a smooth and rough strain of *Bact. phaseoli sojense*. SHARP reports isolation from a culture of *Bact. phaseoli sojense* of a strain whose colonies on agar media are smooth, and another whose colonies are rough. These, in harmony with the practice of animal bacteriologists, were designated as smooth (*S*) and rough (*R*) respectively. SHARP found further, in harmony with considerable data from the fields of medical and general bacteriology, that smoothness is directly correlated with motility, low agglutinability, and higher virulence, whereas roughness is directly correlated with low or no motility, higher agglutinability, and less virulence. He also found for the first time, in the rough strain, an instance of spontaneous agglutination of a schizomycete in distilled water. Finally, in harmony with data relative to some animal pathogenes, he found that high agglutinability was directly correlated in the acid range with low electricphoretic potential (P.D.), and low agglutinability with a high P.D. In preliminary experiments he, and later in more detailed work FALK, SHARP, and LINK (2), found for the first time that this correlation between agglutinability and P.D. does not exist in the alkaline range for either the smooth or the rough strain.

This paper is a report of data which have a bearing upon this interesting and apparently important discovery by SHARP. A discussion of the full significance of these phenomena in the light of data

from the fields of general and medical bacteriology is reserved for a later and more detailed paper.

The original sources of the cultures used are: *Bact. citri* (E. F. SMITH, 1927), *Bact. medicaginis* var. *phaseolicola* (W. H. BURKHOLDER), *Bact. phaseoli sojense* (*R*) (SHARP, 1926), and *Bact. tumefaciens* (A. J. RIKER, 1925). The immediate source of the cultures of *Bact. tumefaciens* were isolations from galls produced upon inoculation of tomatoes by Miss HULL with a culture which SHARP had isolated from tomato galls following inoculation with the original culture.

In the course of routine classwork with *Bact. tumefaciens*, Miss HULL found it impossible to follow SMITH's (12) advice to select only colonies which come up smooth and glistening and remain translucent. She obtained colonies which were opaque and wrinkled at first, and only later became smooth and glistening. Such colonies upon inoculation of tomato plants consistently developed typical galls. She and other students also had found that cultures from such colonies gave the cultural tests characteristic of *Bact. tumefaciens*. This experience was forgotten until agglutination tests were made by LINK and LINK (6) and LINK and TALIAFERRO (7), in which *Bact. tumefaciens* antiserum and suspensions of this organism were used. The first tests were run with the normal serum of animals selected for immunization. An even suspension of *Bact. tumefaciens* had been obtained following three washings in 0.85 per cent NaCl solution and centrifugation for 45 minutes at 3000 revolutions per minute. When the agglutination tests were read, it was found that while there had been flocculation in all tests at all dilutions in which *Bact. tumefaciens* was the test antigen, most of the suspended organisms still were in suspension. The controls in saline solution had behaved identically. Fortunately some of the excess stock suspension of *Bact. tumefaciens* had been kept in the ice chest over night, and it was found that in this, too, there had been beautiful flocculation of a fraction of the organisms (about one-fourth), and that the remainder were still in suspension. In reflecting upon the situation and casting about for a possible explanation, the experience of Miss HULL was recalled in the light of SHARP's findings.

It was thought possible that we were again dealing with an organism which showed the phenomenon of smooth and rough colo-

nies. More detailed work growing out of this working hypothesis has revealed that on potato-dextrose or beef-dextrose agar a culture of the strain of *Bact. tumefaciens* of tested pathogenicity comes up in colonies, about 50 per cent of which are either entirely wrinkled and opaque or only partly so, while the other half consists of smooth translucent colonies (figs. 1, 2). However, the rough colonies develop smooth shiny translucent margins, and after three days on potato-dextrose agar, and after five days on beef-dextrose agar, the colonies appear smooth and shiny and translucent as described by SMITH, although the opaque centers remain visible (fig. 3). On agar

FIG. 1.—Typical smooth surface colonies of *Bact. tumefaciens* on beef-dextrose poured plates, six days old; ×2.

slants the *S-R* cultures remain rough at the margins where new growth is most intense for four or five days before becoming entirely smooth. The rough colonies are very compact and firm, so that when transfer of a portion of them is attempted by needle insertion, the entire colony usually is lifted from the agar surface. Upon similar procedure with a smooth colony only a slight amount of the colony adheres to the needle.

In the course of the tests it was noted that colonies of *Bact. citri* and *Bact. medicaginis* var. *phaseolicola* developed what appeared to be roughness. In shake dilutions of *Bact. citri* in beef-dextrose agar or in potato-dextrose agar colonies appear which are smooth and rough, but more rough than smooth, and which become smooth ulti-

mately. On potato agar the colonies are more rough than on beef agar. These colonies, however, are not as strikingly rough as those of *Bact. phaseoli sojense* or *Bact. tumefaciens*, and appear more like those described by SMITH as having internal convolutions.

Bact. medicaginis var. *phaseolicola* (BURKHOLDER'S no. 35) always gave smooth colonies. Strain no. 23, however, which BURKHOLDER considered identical with no. 35, develops smooth-rough colonies on potato-dextrose and beef-dextrose agar. BURKHOLDER (1) describes this organism as having colonies on nutrient agar that are concentrically ringed with "edges undulate." On gelatin the

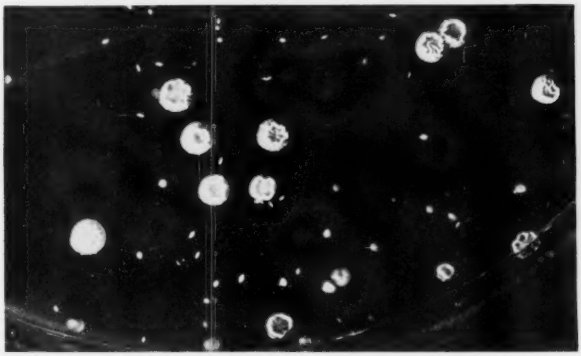


FIG. 2.—Typical rough colonies of *Bact. tumefaciens*, four days old; ×2

colonies are raised and "somewhat wrinkled." The colonies are butyrous at first but later become brittle. *Bact. phaseoli sojense* (*R*), in contrast with *Bact. citri* and *Bact. tumefaciens*, comes up smooth and becomes and remains rough on both potato-dextrose and beef-dextrose agar.

These observations would seem to indicate that smoothness and roughness are not absolutely qualitative differences, but that they have a quantitative element in them. Apparently there are intergrades, of which perfect smoothness is one extreme and extreme roughness the other. This is borne out by data relative to the agglutinability of the organisms under discussion.

SHARP found that the *R* strain of *Bact. phaseoli sojense*, which consists of extremely rough but butyrous colonies, agglutinated

spontaneously in both distilled water and in 0.85 per cent NaCl solution, and that nine washings in distilled water did not render it suspensible. LINK and LINK (**6**) three months later, dealing with subcultures from the same culture, found that the organism still agglutinated spontaneously in 0.85 per cent NaCl solution, but that after resuspension and washing, a permanent suspension could be obtained which was used by them in agglutination tests. This may indicate that the organism is in an unstable condition at present, and that reversion may be taking place.

Bact. citri and *Bact. medicaginis* var. *phaseolicola* no. 23, which develop partially rough colonies, when suspended in 0.85 per cent

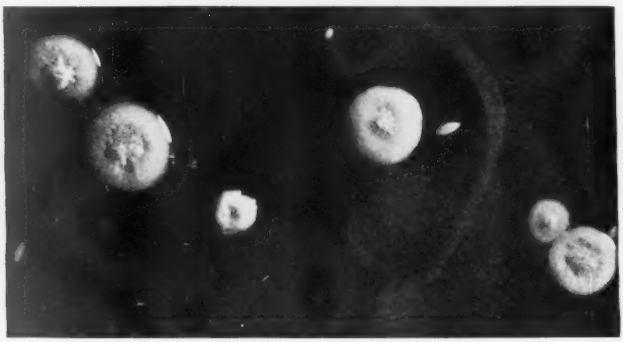


FIG. 3.—Transition colonies of *Bact. tumefaciens*, showing rough center and smooth margin, six days old; ×2.

NaCl solution do not settle out, even after 36 hours. On the other hand, *Bact. malvacearum*, *Bact. phaseoli*, *Bact. flaccumfaciens*, *Bact. phaseoli sojense* (*S*), and *Bacillus carotovorus* (*Iris*), which show no evidence of roughness in the cultures, settled out slightly from suspensions after 24 hours, while suspensions of the smooth organisms *Bact. campestre*, *Bact. tumefaciens*, *B. carotovorus* (3*a*), and *B. aroideae* settled out heavily. These data indicate that apparently there is not always a definite direct correlation between roughness and ready flocculation, and smoothness and less ready agglutination. Further work may show, however, that there are various types of roughness and smoothness, and that agglutinability is correlated with a definite type of roughness.

Bact. tumefaciens seems to be the most unstable organism so far studied. Interest in this apparent instability is enhanced by the discovery, by LINK and LINK (**6**) and LINK and TALIAFERRO (**7**), of what may prove to be a case of serological cosmopolitanism when antisera of various organisms are tested against suspensions of *Bact. tumefaciens*. *Bact. tumefaciens*, like *Bact. phaseoli sojense* (*R*), agglutinates spontaneously in distilled water. Suspensions in distilled water of colonies which appear entirely (or mostly) rough give almost complete clearing of the supernatant liquid in 12 hours, while suspensions made from older colonies which appeared smooth had many organisms in suspension after 12 hours, although there also was a heavy precipitate.

Whether such correlation between character of colony agglutinability and virulence exists for these organisms, as has been described by SHARP for *Bact. phaseoli sojense*, remains to be determined. Investigations are under way on this problem. Because of the possibility that in *Bact. tumefaciens* we are dealing with an organism in which an intermediate form is changing into the *R* and *S* forms, or the *R* form is giving rise to an intermediate or smooth form, the results of these studies are awaited with much interest.

SMITH (**12**) has used pathogenicity and production of specific symptoms as important criteria for species identification. Serological work (**5, 6, 7, 10**) with pathogenes which are not readily differentiated culturally, but which show host specificity, indicate strongly that SMITH was correct in his theory. In the light of these facts and the data here reported for *Bact. tumefaciens*, the directions which SMITH has given for isolation of this organism are very significant. He states:

> . . . . and all circular white colonies, if opaque, are negligible. Only those colonies that come up slowly, that remain for a considerable time small, circular, raised and glistening-translucent (watery) need be considered, and. . . . . Some colonies selected for the subcultures may not prove to be infectious . . . . it is advised to experiment with quite a number of colonies, rejecting all that show a narrow clear zone about the colonies.

He also refers to the observation that colonies with a thin, wrinkled, and dull surface develop on agar after passage of the organism through peptone-bouillon.

There are other references in the literature which indicate that *Bact. tumefaciens* is unstable, both as to form and virulence. SMITH, BROWN, and TOWNSEND (11), RIKER (8), and ROBINSON and WALKDEN (9) ascribe various sizes to *Bact. tumefaciens*. SMITH has repeatedly referred to involution forms:

> It passes over easily (under action of cold, sodium chloride, or acids) into club-shaped, Y-shaped, and variously branched involution forms, which often are dead or dying, i.e., will not grow on agar-poured plates, or come up slowly. These moribund involution forms occur not only in culture media, but are common in the tumor, and to them must be attributed not only our former difficulty in isolating the organism, but also the failure of others to isolate.

LEVINE (4) goes a great deal further, and reports decided changes in form of *Bact. tumefaciens* which suggest the phenomenon of cyclogeny. He refers to the appearance of jelly-like substances of amorphous form which appear in old cultures, and which upon transfer to new media give rise to new growth in which long rods develop, some of them of beaded appearance. The rods are reported to break up, until finally small faintly staining cocci with bacilli or filaments appear. The cocci finally give way to the amorphous masses first described.

One is moved to query how many other similar phenomena might be discovered if all plant pathogenes were studied as critically as *Bact. tumefaciens* has been. There are some references to colony instability (not so termed in the literature) which indicate that the phenomenon probably is as frequent among plant pathogenes as it is among the forms studied by the general and medical bacteriologist. SMITH's description and illustrations of the "windowed" colonies of *Bact. malvacearum* are significant. They may represent erosion phenomena. The colonies pictured in fig. 250, to show the effect of freezing, look as rough as the rough colonies of *Bact. phaseoli sojense*. The remarkable series of photographs of colonies of *Bact. translucens* var. *undulosum* showing liquefaction pits are very suggestive of lytic phenomena. GARDNER and KENDRICK (3) describe aberrant colonies of *Bact. viridifaciens*, and illustrate one with lobed margin and surface sculpturing.

Conclusions

1. The phenomenon of roughness and smoothness of colony form is reported for *Bact. citri*, *Bact. medicaginis* var. *phaseolicola* no. 23, and *Bact. tumefaciens*.

2. These organisms seem to be in a state of instability so far as these characters are concerned, *Bact. tumefaciens* seeming to be the least stable.

3. In *Bact. tumefaciens* roughness in colony form seems to be correlated directly with ready spontaneous agglutination in distilled water, and in 0.85 per cent NaCl solution, whereas smoothness seems to be directly correlated with less agglutinability. Mixed cultures can be separated roughly by means of this differential agglutination.

4. These phenomena of colony form and agglutination seem to be qualitative and quantitative or perhaps merely quantitative.

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[*Accepted for publication March 16, 1927*]

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CYTOLOGICAL STUDY OF STIGONEMA MAMMILOSUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 371

SYBEL LEE

(WITH PLATE XIII)

The Cyanophyceae, whether considered as the lowest of the algae, or as closely related to the bacteria, are interesting on account of primitive features which might give investigators a better understanding of the cells in higher plants; while the central body might help to solve the problem of the origin of the nucleus.

In 1904–1905 OLIVE (5), who studied *Oscillatoria* and other species of Cyanophyceae, came to the conclusion that the central body is a nucleus with mitotic division not essentially different from that in higher plants. GARDNER (1) reported that the Cyanophyceae contain a nucleus which occupies a relatively large portion of the cell, but which divides amitotically, except in *Synechocystis*, which possesses a primitive form of mitiosis. HAUPT (2), studying *Anabaena*, came to the rather surprising conclusion that the cell of the Cyanophyceae has no real nucleus, although the central substance resembles chromatin and may have similar functions.

Although the Cyanophyceae have been investigated exhaustively by many cytologists there is no general agreement. Since *Stigonema* was available and had received little more attention than a taxonomic treatment, Professor CHARLES J. CHAMBERLAIN, to whom I am indebted for advice and criticism, suggested that I examine this genus.

Material and methods

Material was collected in 1920 at Mount Desert, off the coast of Maine. It was fixed in a weak Flemming solution and was brought up to the 70 per cent alcohol by Dr. WM. R. TAYLOR, who identified the species as *Stigonema mammillosa*. Since *Stigonema* is a simple plant, material mounted whole will show the general features; but sections cut from 3–8 μ are better for details.

The Venetian turpentine method was used for the whole mounts. Iron-alum haematoxylin is good for cell contents, but crystal violet is a better stain for the sheath. For sections, safranin followed by Orange G in clove oil was tried, but was not as good as iron-alum haematoxylin for the differentiation of cell contents. Crystal violet was also used in sections for differentiating the sheath.

Topography

Taxonomically *Stigonema* is classified under the filamentous forms with true branching, but the cells are aggregated more or less like colonies. In the same filament each group has cells in different stages, so that their appearance in the living condition and their reactions toward the stains are different, some groups of cells staining deeply while the others stain very lightly, producing a spotted appearance.

There is true branching, but it is irregular (figs. 1, 2). The smallest branches are only one cell in thickness (fig. 3), while the largest reach ten cells in diameter. The cells of the young branches always stain alike, as shown in figs. 3 and 4; consequently these cells are more or less in the same stage. Some of the cells in the older filaments become rejuvenated while the rest remain old, so that there are different conditions in the same filament. The outer cells are most likely to rejuvenate and give rise to new branches, while the rejuvenation of inner cells gives rise to new groups. In the limited space and the struggle for existence the weakest cells and groups of cells degenerate (figs. 3, 6). The vigorous groups of cells, abundantly supplied with chlorophyll and phycocyan, contrast sharply with the weaker and degenerating cells, and thus cause the spotted appearance which gives the genus its name.

A thick gelatinous mass, the sheath, envelops the cells, except in very young filaments where the cells are in close contact with one another, so that the whole plant is much like a loose association of colonies. The sheath about single cells and groups of cells is often distinctly lamellated.

There are four kinds of cells: the heterocyst (fig. 2), concave cells (fig. 2), degenerating cells (figs. 3, 6), and the active vegetative cells.

Vegetative cells

The vegetative cells of *Stigonema* are spherical in shape, because they are free from pressure of neighboring cells, on account of the intrusion of gelatinous secretions. The cells are almost as free from one another as if the plant were unicellular; however, the cells of each group are connected by protoplasmic connections, which are often quite conspicuous (fig. 7).

There are no chromatophores, the protoplasm occupying the entire space between the central body and the cell wall. It is differentiated into two regions, a peripheral and an inner region, the former darker than the latter, because of the coloring matters (figs. 5, 6, 8). The protoplasm of the resting cells is more or less vacuolated, but that of the active cells seems nearly homogeneous.

The so-called central body is a nucleus. It occupies a relatively large portion of the cell, and is sharply marked off from the protoplasm in spite of the absence of a nuclear membrane (fig. 8). It is composed of an achromatic portion and chromatic granules. The chromatic portion is composed of an unusually dense substance, consisting of a more or less definite number of chromatic granules (figs. 8, 9), which become aggregated into rodlike bodies resembling chromosomes when the nucleus divides (figs. 10–15). Two groups of these bodies are formed, but they do not split lengthwise and divide equally like chromosomes in the higher plants.

While the central body is a naked nucleus, it should be regarded as the homologue of the true nucleus of the higher plants. It is more primitive and does not show such a precise division and distribution of the chromatic material, but there is an aggregation of chromatic granules to form a spireme stage, which is followed by a segmented stage (figs. 10–12). The spireme and the segmentation resemble somewhat the behavior of the nucleus as described by KOHL (4) for *Tolypothrix;* but the spireme is not so complete nor distinct as shown in his figures. There is also some resemblance to the figures in HAUPT'S paper on *Anabaena*, where we should interpret the deeply staining strands in the central body as chromatin. The arrangement of these segments into two groups follows, and cell division takes place (figs. 13–16).

Nothing was found which might be identified as a spindle or spindle fibers, nor was any formation of a cell wall discernible in the region where a spindle might be anticipated; but after nuclear division the elongation of the cell takes place, and is followed by the appearance of a cleavage furrow which becomes deeper and deeper until the cell itself is separated into two. Nuclear division always precedes the appearance of cleavage, because when the chromatic groups separate they leave a less resistant region in the outer wall, which gradually presses in until the daughter cells are formed. After that the nuclei of the daughter cells break up into chromatin granules (figs. 17, 18).

The cells of the resting stage contain a great number of accumulated food granules, which will be used when the cells are rejuvenated. The chromatic substance of the central body becomes condensed into a single deeply stained body suspended by its radiations in the center of the cell (figs. 19, 20). No granules could be distinguished in this dense body, which looks as homogeneous as the nucleus of *Porphyra*, as figured and described by ISHIKAWA (3).

Conclusions

1. The cells of *Stigonema* act independently, although aggregated into a filament which is often many cells in diameter.

2. When a cell is rejuvenated at the surface of the filament it produces a true branch; when the rejuvenating cell is more deeply placed it produces a group of vigorous cells, which, being surrounded by comparatively inactive cells, gives the filament a spotted appearance, which suggested the generic name.

3. The central body is a primitive nucleus which has no nuclear membrane or nucleolus, and no spindle during division.

[*Accepted for publication January 27, 1927*]

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DESCRIPTION OF PLATE XIII

FIG. 1.—Portion of filament mounted whole; ×65.

FIG. 2.—Old branch with three young branches: *c*, concave cell; *h*, heterocyst; ×65.

FIG. 3.—Adult branch with young branch and group of cells from a rejuvenating cell (*r*); ×532.

FIG. 4.—Young branch somewhat older than those in figs. 2 and 3; ×532.

FIG. 5.—Transverse section of adult branch with young one; ×532.

FIG. 6.—Adult branch with numerous degenerating cells; ×532.

FIG. 7.—Two cells, lower showing central body with radiations and upper showing connection between cells; ×1080.

FIG. 8.—Two cells, upper showing central body with radiations and chromatin granules; ×1080.

FIG. 9.—Several cells showing aggregation of granules; ×1080.

FIG. 10.—Aggregation of chromatin granules into rodlike chromat in masses; ×1080.

FIGS. 11, 12.—Cells with nucleus showing rodlike chromatin masses; ×1080.

FIG. 13.—Cell with chromatin beginning to divide into two groups; ×1080.

FIG. 14.—More advanced stage in division; ×1080.

FIG. 15.—Chromatin in two groups and furrows dividing the cells; ×1080.

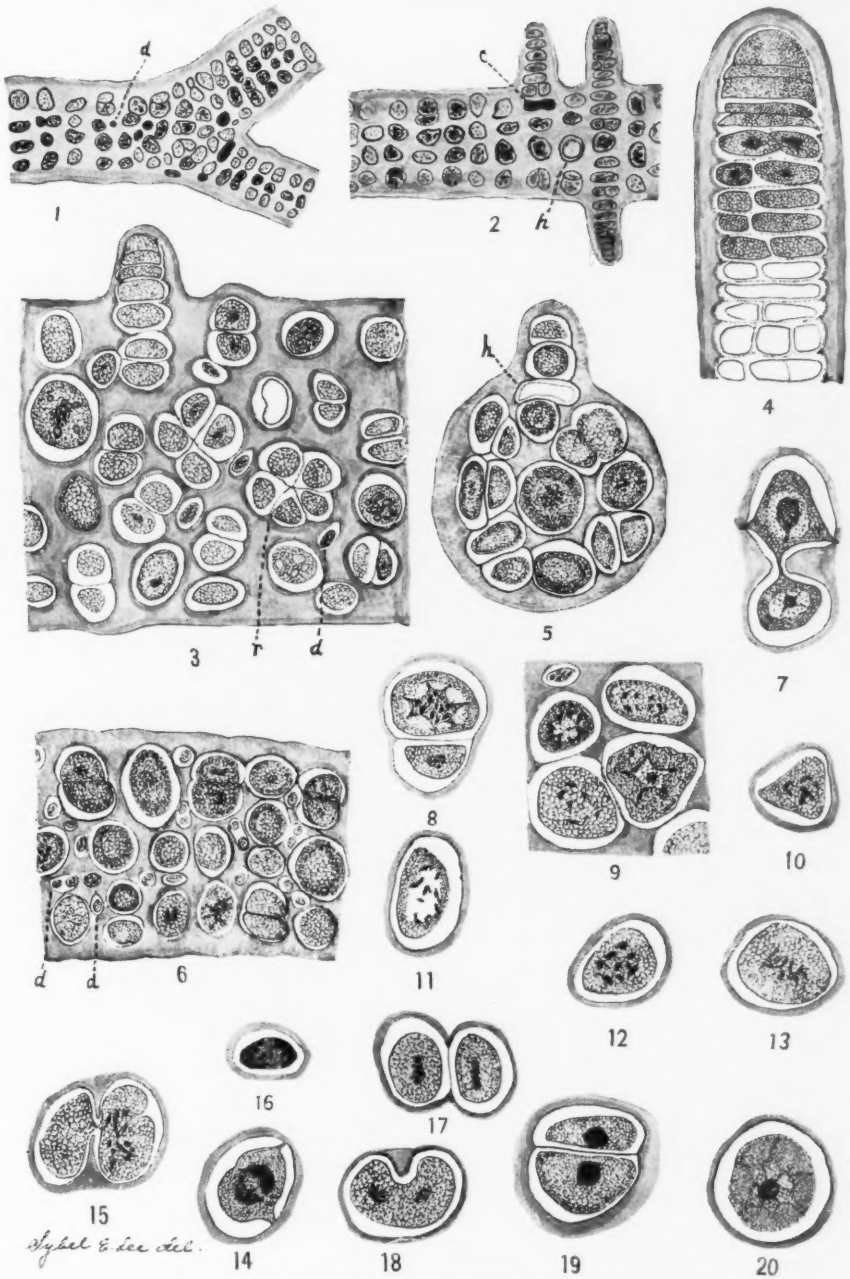
FIG. 16.—Advanced stage in nuclear division; ×1080.

FIG. 17.—Division completed; ×1080.

FIG. 18.—Two groups of chromatin and beginning of cleavage furrow; ×1080.

FIG. 19.—Division completed and chromatin in homogeneous mass; ×1080.

FIG. 20.—Nucleus with homogeneous chromatin and radiating strands; ×1080.



LEE on STIGONEMA

BRIEFER ARTICLES

ISOSTIGMA PEUCEDANIFOLIUM (SPRENG.) LESS., A VALID NAME

In a former article (BOT. GAZ. **81**:241–257. 1926) I presented a revision of the entire genus *Isostigma* Less. At that time an extended discussion of *I. peucedanifolium* (Spreng.) Less. was given (*l.c.* pp. 252–254). A specimen borrowed from the Delessert Herbarium, and which had seemed to be authentic for *Bidens megapotamica*, was discussed in detail. It was stated that this specimen was of the species widely known as *Isostigma peucedanifolium*. As my repeated searches in Paris for the real type of *B. megapotamica* Spreng. all had ended in failure, it seemed probable that no type had been preserved among SPRENGEL'S specimens, and that therefore the apparent cotype in the Delessert Herbarium would have to suffice. Under the compulsion incident to giving a monographic treatment of the genus the trivial name *megapotamica* was adopted as the earliest for *Isostigma peucedanifolium* (Spreng.) Less., and the resulting new combination *Isostigma megapotamicum* was employed.

In the spring of 1926, I was submitted for advance perusal and criticism some preliminary pages of manuscript upon the genus *Thelesperma*, by S. F. BLAKE, Associate Botanist of the United States Bureau of Plant Industry. It was a pleasant surprise to find at one point in the reading of his treatment that BLAKE had actually found the type itself for *Bidens megapotamica* Spreng. While searching at Paris (Herb. Mus. Hist. Nat.) for various Compositae in other genera, he had found this type, mounted upon the same sheet with the type of *Tagetes flosculosa* Spreng. Of these two specimens he obtained a small but excellent photograph. This illustration confirms BLAKE'S statement (*in. litt.*) that the type of *B. megapotamica* Spreng. "is a plant which is clearly *Thelesperma scabiosoides*." This being the case, the name *megapotamica* belongs in *Thelesperma*, where the combination *Thelesperma megapotamicum* (Spreng.) O. Ktze. must stand, and the name *T. scabiosoides* Less. must reduce to a synonym, as stated once before (BOT. GAZ. **76**:91. 1923). *Isostigma peucedanifolium* (Spreng.) Less. must remain, therefore, the valid name for the species numbered 9 in my Revision (*l.c.* 252).—EARL EDWARD SHERFF, *Chicago, Ill.*

CURRENT LITERATURE

BOOK REVIEWS

Photosynthesis

A very valuable summary of the facts now known about photosynthesis, and the theories proposed to account for it, has been prepared by SPOEHR.[1] There are seven chapters in the book, with general headings as follows: Origin of organic matter and the cosmical functions of green plants; Nature of photosynthesis as determined by observations of gaseous interchange and the origin of organic matter; Products of photosynthesis; Methods of measuring photosynthetic activity; Chemistry of photosynthesis; Energy relations in photosynthesis; and Chlorophyll and chloroplasts.

The first chapter particularly is of general interest, and should be read by every teacher of botany and by every student who wants to know something of the significance of botanical science. The succeeding chapters are somewhat more technical, of course, but the book is quite readable to those who are familiar with organic chemistry; and it will no doubt stimulate a great deal of interest in the unsolved problems connected with the synthetic activities of plants. To students and workers especially interested in the physiology of plants, the chapters on the products, methods of measuring, and the chemistry of the process will be most attractive.

The chapter on energy relations is based largely upon the work of BROWN and his coworkers ESCOMBE and WILSON. Unfortunately for the work of these pioneers in the study of energy relations, excellent as it seems to be on first examination, they considered the reflection of light from leaves negligible, and worked out their ideas of the quantitative utilization of sunlight energy by the leaves of plants from this point of view. The reviewer has been engaged recently in measuring the reflection of energy in the form of light from the surfaces of leaves, and finds that a much larger proportion of the energy than is usually assumed may be reflected from the surfaces of leaves. In the darker portions of the spectrum, the shortest violet and longest red rays, the reflection may run as low as 3 per cent, but in the brightest green region of the spectrum, depending upon the individual leaf color, the reflection of light at an angle normal to the surface is seldom less than 8 per cent, and may run as high as 20 per cent in light green vegetation such as is seen in early spring. Autumn colored leaves and albino leaves reflect still larger percentages of the incident energy, even exceeding 50 per cent in some cases. These results throw grave doubts upon the accuracy of the BROWN and ESCOMBE figures, in which they worked out a 100 per

[1] SPOEHR, H. A., Photosynthesis. 8 vo. pp. 393. Chemical Catalog Co. New York. 1926.

cent balance sheet of the energy income and outgo of leaves, on the supposition that reflection is negligible. This chapter, then, deals with a phase of the subject which needs reinvestigation, and a rewriting *in toto*. The fundamental facts are needed before we can draw a reasonable picture of the energy relations of the plant.

The book is so well written, and the materials so admirably marshalled that one dislikes to point out errors of judgment. However, there is one feature in chapter II which calls for comment. In connection with gaseous exchange the author considers the photosynthetic quotient, and incidentally the respiratory quotient of plants. He points out the confusion in the literature over the matter of designating these ratios, and then adds to the confusion by choosing the wrong designation. The animal physiologists have for a long time used $\frac{CO_2}{O_2}$ to designate the respiratory ratio, and this has been at least common among plant physiologists. There is no indication that the animal physiologists will ever change their method, and they consider it rather absurd to write the ratio $\frac{O_2}{CO_2}$. There are many reasons why plant and animal physiologists should use common symbols for the processes that are common to plants and animals, as respiration is. The reviewer considers it an unfortunate error in judgment to use $\frac{CO_2}{O_2}$ as the photosynthetic quotient, and $\frac{O_2}{CO_2}$ as the respiratory quotient. Certainly this method will not be followed by animal physiologists, and it ought not to be by plant physiologists.

A few typographical errors occur, as one might expect to find even in the best of books. The most striking instance noted by the reviewer in reading the book was on page 131, where the word calorie occurs as "callory" and "calory" in consecutive lines. If the book should reach a second printing a number of errors, including these, should be eliminated.

Taking the book as a whole, it is one of the best American monographs so far published in the field of plant physiology. It is hoped that the reception given it will encourage the preparation of other valuable monographs in this field.—C. A. SHULL.

Surface chemistry

The rapid development of the field of surface phenomena is emphasized by the appearance of a book by RIDEAL[2] on surface chemistry. The reactions, the equilibrium relations, and the chemical structure of interfaces must be thoroughly understood before we can grasp the essential features of adsorption, enzyme and other catalyses, and the causes of stability in colloidal gel and sol systems. Beyond this are the phenomena of life, anabolic and catabolic changes, the

[2] RIDEAL, E. K., An introduction to surface chemistry. 8vo. pp. x+336. Cambridge University Press. Cambridge, England.

effects of anaesthetics and other surface-active chemicals on the living colloidal systems of organisms. These phenomena can only be understood properly in the light of interphase chemistry and physics.

The book by RIDEAL draws together the facts concerning the molecular structure and kinetics of the two dimensional world represented by phase surfaces, as developed by the investigations of such pioneers as Lord RAYLEIGH, MARCELIN, HARDY, LANGMUIR, and others. The author, as a follower in the footsteps of these earlier students of surface phenomena, has already contributed in excellent fashion to the advancement of this field. He sets forth the discussion in nine chapters, which take up the surface tension of liquids, surface tension of solutions, surface films of insoluble materials, liquid-liquid interfaces, the gas-solid interface, liquid-solid interface, difference of potential at interfaces, conditions of stability in suspensions and emulsions, gels and hydrated colloids.

The first seven chapters lay down an admirable basis for the understanding of the last two chapters, which will be most interesting to students of biology. In the chapter on stability of suspensions and emulsions there is a good discussion of Brownian movement, electrolytic coagulation, adsorption of ions, and rules of precipitation of colloids. The effects of non-electrolytes on suspensions, and the precipitation of metallic sols by minute quantities of protective colloids are given brief treatment at the close of this chapter.

The final chapter on gels and hydrated colloids considers the theories of gel structure, structure of gelatin, rigid and moist gels, and the properties of such gels as silica gel, gelatin, soaps, and colloidal dyes. As DONNAN, who writes the preface, remarks, "every student and investigator of surface and colloid phenomena owes Dr. RIDEAL a warm debt of gratitude for his admirable survey and presentation of a great and rapidly advancing field of physico-chemical science." —C. A. SHULL.

Economic botany

One of the most attractively written books on botany that the reviewer has ever read is one by PEATTIE[3] on the important crop plants of the world. The volume might well be styled romantic tales of economic plants. Starting with the rather well known history of the spice trade, the author tells in lucid language of the important part played by many crops in the history of man. Suggestive chapter titles are: quinine, the coming of a savior; the age of rubber; camphor, the strategic crop; the potato, the poor man's friend; the poppy, blessing and curse; tobacco, the companionable weed. It is shown quite conclusively that plants quite as much as other things have determined the rise and fall of nations. In the final chapter, entitled "Must we starve?", it is shown that the key to the future of mankind resides in plants. To one who does not believe in the significance of botany, this book must indeed be a potent revealer of new truth. The figures are quaint maps depicting modern facts in the garb of the middle ages.—H. C. COWLES.

[3] PEATTIE, D. C., Cargoes and harvests. 8vo. pp. 311. *figs. 16*. New York: D. Appleton and Co. 1926.

NOTES FOR STUDENTS

Taxonomic notes.—RILEY[4] has described 11 new species secured by a collection made in Panama in 1924. The collection was made within 10 miles of the city of Panama, and, as the author says, "it is somewhat notable that so many new species (8) should have been obtained in what is now a well worked area botanically." The new species belong to the following genera: *Odontocarya* (Menispermaceae), *Elsota* (Polygalaceae), *Erythrochiton* (Rutaceae), *Eugenia* (Myrtaceae), *Alibertia* (Rubiaceae), *Psychotria* (Rubiaceae), *Rauwolfia* (Apocynaceae), *Cordia* (Boraginaceae), *Solanum* (Solanaceae), and *Acalypha* (Euphorbiaceae).

SPRAGUE and SANDWITH[5] have described 7 new species of *Strychnos* from Tropical America. The genus is said to include several hundred species "described and undescribed." It occurs most abundantly in Brazil and Guiana. The species described in this paper are from Mexico, Venezuela, Peru, and Brazil.

In an account of the glabrate species of *Tilia*, BUSH[6] has described a new species (*T. Ashei*) from Florida. The glabrate group includes 11 species and 6 varieties, 11 of which are referred to SARGENT, with some changes in classification.

GLÜCK[7] has described a new species of *Sagittaria* (*S. Kurziana*) from northwestern Florida.

MERRILL[8] has described 21 new species of ligneous plants from China, representing as many genera. *Bennettiodendron* is a new generic name, substituted for *Bennettia* Miquel.

CROW[9] has described a new genus (*Crinalium*) of Cyanophyceae, belonging to the Oscillatoriaceae. It was found in North Wales on the damp face of a rock, the filaments being endophytic in gelatinous colonies of a species of *Aphanocapsa*.

FERNALD[10] has published an account of his experiences in botanizing in Newfoundland for two summers. Many plants new to the flora either of Newfound-

[4] RILEY, L. A. M., New species from Panama, and Coiba and Cocos Islands. Kew Bull. Miscell. Inform. no. 3. 119–127. 1927.

[5] SPRAGUE, T. A., and SANDWITH, N. Y., New species of *Strychnos* from Tropical America. Kew Bull. Miscell. Inform. no. 3. 127–133. 1927.

[6] BUSH, B. F., The glabrate species of *Tilia*. Bull. Torr. Bot. Club **54**:231–248. 1927.

[7] GLÜCK, H., A new *Sagittaria* from Florida. Bull. Torr. Bot. Club **54**:257–261. 1927.

[8] MERRILL, E. D., New Chinese ligneous plants. Jour. Arnold Arboretum **8**:3–19. 1927.

[9] CROW, W. P., *Crinalium*, a new genus of Cyanophyceae, and its bearing on the morphology of the group. Ann. Botany **41**:161–165. 1927.

[10] FERNALD, M. L., Two summers of botanizing in Newfoundland. Contrib. Gray Herb. 76. pp. 144. 1927.

land or of Eastern America were discovered. The collections are listed, together with some revisions. There are given also full citations of collections elsewhere, thus indicating the geographical distribution. There are 13 new species described, 3 of which belong to *Salix*, and also 9 new varieties.

JOHNSTON,[11] in continuation of his studies of the Boraginaceae, has published a revision of the American Boraginoideae known from south of Panama. There are 17 genera presented, one of which (*Nesocaryum*) is described as new, and 86 species. Much the largest genera are *Cryptantha*, with 40 species, and *Plagiobothrys* with 22. There are 11 new species described and many new combinations.

RYDBERG,[12] in continuation of his studies of the Fabaceae, has published an account of *Hamosa*, a genus of 20 species, 13 of which he has segregated from *Astragalus*, and 4 of which are described as new.

RIDLEY,[13] in concluding his presentation of *Argostemma*, describes 15 new species and 4 new varieties, and also establishes a new genus, *Argostemmella*, with 2 species from Borneo.—J. M. C.

Biological Abstracts.—The first number of this publication has appeared, bearing the date December 1926. It contains abstracts of 1878 titles, representing the whole field of the "World's literature in theoretical and applied biology, exclusive of clinical medicine." Of the 1878 titles, 63 are general, 109 are in bacteriology, 700 are in botany, and 1006 are in zoology. The subjects covered in the more general field are biography, history, and bibliography, general biology, evolution, cytology, genetics, biometry, and ecology. The subjects in the field of plant science are phytopathology, plant physiology, biochemistry and biophysics, systematic botany, morphology and anatomy of vascular plants, paleobotany, pharmacognosy and pharmaceutical botany, forestry, horticulture, and agronomy. The organization of the material is quite complete, so that papers and authors are very accessible. As stated in the preface, the necessity for such a guide to current literature is due to "the increasing interdependence of the various fields of biological science, coupled with the constantly growing volume and complexity of the literature." This publication should certainly be accessible to every botanist. It will appear monthly, the volume closing the calendar year with a concluding index. This is a great cooperative enterprise, and biologists throughout the world are expected to contribute abstracts. Although the subscription price is $15.00 a year, a special price of $9.00 is made for all personal subscriptions. The editorial and executive office is located at the University of Pennsylvania, Philadelphia.—J. M. C.

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[11] JOHNSTON, I. M., A revision of the South American Boraginoideae. Contrib. Gray Herb. 78. pp. 118. 1927.

[12] RYDBERG, P. A., Notes on Fabaceae. VIII. *Hamosa*. Bull. Torr. Bot. Club **54**: 13–23. 1927.

[13] RIDLEY, H. N., The genus *Argostemma*. Jour. Bot. **65**:33–41. 1927.

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THE UNIVERSITY OF CHICAGO PRESS

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